Dissertation on

A CLINICAL STUDY ON THE STATUS OF THE ENDOTHELIUM AFTER DEEP ANTERIOR LAMELLAR KERATOPLASTY

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BRANCH - III

Regional Institute of Ophthalmology

Madras Medical College & Research Institute,

Chennai – 600 003

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 003

TAMIL NADU

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CERTIFICATE

Certified that this dissertation entitled "**A CLINICAL STUDY ON THE STATUS OF THE ENDOTHELIUM AFTER DEEP ANTERIOR LAMELLAR KERATOPLASTY**" is the bonafide work by **Dr. SOUMYA RAMANI** , Post graduate student, done under my guidance and supervision during the period from June 2007 to November 2009 in partial fulfillment for the award of M.S. Degree (Ophthalmology) of the Tamil Nadu Dr. M.G.R. Medical University, Chennai.

Prof. Dr.K Vasantha, M.S., F.R.C.S.,
Professor,
Head of the Dept, Cornea Services,
Regional Institute of Ophthalmology,
Government Ophthalmic Hospital,
Chennai - 8.

Prof.Dr.M.Radhakrishnan, M.S., D.O.
Director & Superintendent,
Regional Institute of Ophthalmology,
Government Ophthalmic Hospital,
Chennai-8.

Prof. Dr Mohanasundaram, M.D, PhD, DNB,
Dean,
Madras Medical College,
Chennai

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INTRODUCTION

Arthur von Hippel performed the first LKP in the last quarter of the 19th century. The basic principle is to replace only that part of the cornea that is diseased and leave the recipient's normal corneal layers intact.[1,2] The idea is to to do the least amount of resection with the greatest amount of benefit thus leaving the healthy endothelium and Descemet's membrane as an immunological barrier to rejection. Deep anterior lamellar keratoplasty (DALK) is a surgical procedure for removing the corneal stroma down to Descemet's membrane.[3] It is most useful for the treatment of corneal disease in the setting of a normally functioning endothelium.

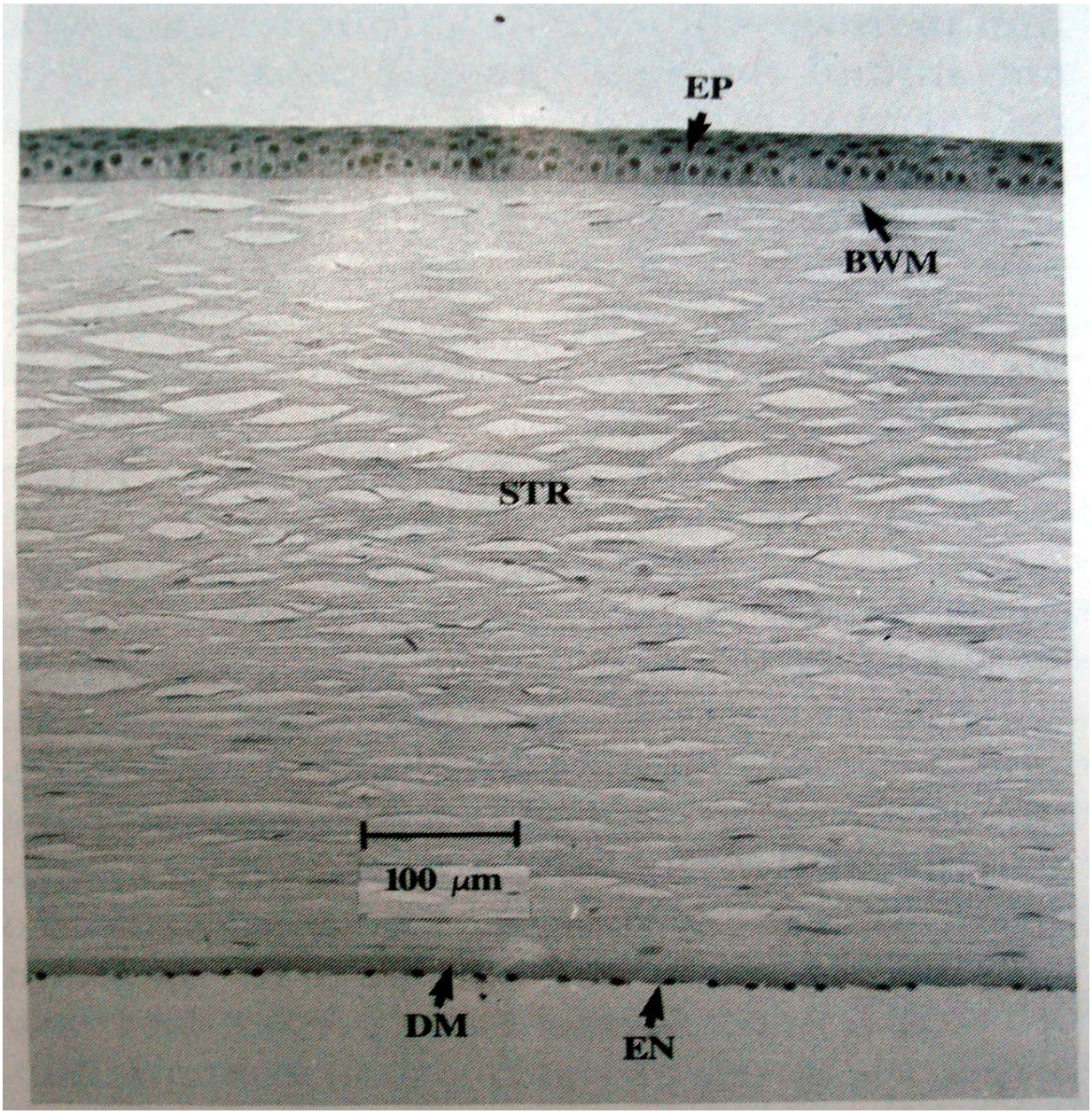
Traditionally, penetrating keratoplasty (PK), which involves a full-thickness corneal graft, has been the treatment of choice for corneal stromal diseases. But PK can be complicated by graft rejection, irregular astigmatism and corneal opacification, thus resulting in visual impairment. DALK offers an alternative procedure that may lessen those risks because the recipient Descemet's membrane and endothelium are preserved. At the same time, DALK carries the potential danger of decreased visual acuity due to possible opacification at the interface layers[3,4]. But, inspite of DALK being a procedure which does not involve manipulation of the anterior chamber, there is a continued endothelial cell loss.

ANATOMY

The cornea is a transparent, avascular watch glass like structure forming the anterior one sixth of the eyeball. It forms the principal refractive surface, contains the intraocular pressure and provides a protective interface with the environment. These functions can be subserved by virtue of a specialized substructural organization.[5]

Histologically, the cornea is composed of five layers:

- Epithelium
- Bowman’s membrane
- Stroma or substantia propria
- Descemet’s membrane
- Endothelium



Photomicrograph of normal human cornea showing the stratified squamous epithelium (EP), Bowman's membrane (BWM), stroma (STR), Descemet's membrane (DM) and endothelium (EN)

1. Corneal epithelium:

It is stratified, squamous and non-keratinized. It is 50 to 90 micron thick and consists of 5 or 6 layers of nucleated cells. The epithelial cells are arranged in three zones:

- Deep zone: It consists of a single layer of basal columnar cells and forms the germinative zone.
- Middle zone: It comprises of 2 to 3 layers of polyhedral cells called wing cells which are convex anteriorly and cap the basal cells.
- Superficial zone: It has 2 to 3 layers of flattened nucleated cells called squamous cells.

2. **Bowman's layer**:

It is a narrow, acellular, homogeneous zone, 8 to 14 micron thick, immediately subjacent to the basal lamina of the epithelium.

It is relatively resistant to trauma due to the compact arrangement of collagen but once destroyed, it can't be regenerated.

3. **Stroma or substantia propria**:

It is around 560 micron thick (90% of the thickness of cornea) and comprises of regularly arranged lamellae of collagen bundles in a proteoglycan ground substance with cells called keratocytes.

The lamellar arrangement is less precise in the anterior stroma.

4. **Descemet's membrane**:

It is a 10 to 12 micron thick basal lamina produced by the endothelium. Its peripheral termination is marked by the Schwalbe's line. The major protein is type IV collagen. Descemet's membrane readily regenerates following injury.

5. **Endothelium**:

The endothelium is the single layer of cells located at the posterior of the cornea; it permits the passage of nutrients from the aqueous humor into the cornea. The requirement for a monolayer that is "leaky" to aqueous humor is met through the barrier function of the endothelium, formed by cell-to-cell contacts and interdigitating lateral membranes. The endothelium is the major cell layer responsible for maintaining the relatively low level of stromal hydration necessary for corneal transparency. Stromal hydration is controlled by the activity of ionic pumps in the plasma membrane of endothelial cells. The relatively high extracellular ion concentration produced by these pumps draws water from the stroma, thus maintaining the highly organized collagen lamellar structure required

for corneal transparency. The endothelium also secretes a thick basal lamina, termed Descemet's membrane, which lies between the endothelial cells and the posterior stroma. Descemet's membrane is one of the thickest basement membranes found in normal tissue; however, its specific function is unknown.[5,6]

Morphologic Characteristics

At birth, the endothelial monolayer consists of about 400,000 cuboidal cells. Each cell is 4 to 6 µm thick, is about 20 µm wide, and has a surface area of about 250 µm2. The average cell density at birth is about 4000 cells/mm2.In young corneas, the endothelium forms a pattern of polygonal cells with five to seven sides. Scanning electron microscopy of the monolayer surface reveals the hexagonal cell shape and numerous lateral, interdigitating cellular processes. These processes increase the area of contact between neighboring cells and resemble interlocking fingers. Occasionally, a centrally located cilium, about 2 to 7 µm long, is present on the surfaces of peripheral cells. The function of this cilium is unclear.

Ultrastructural Characteristics

The ultrastructural features of the endothelium reflect its functions. Numerous mitochondria within the cytoplasm indicate that these cells are

metabolically active. The cytoplasm also contains an elaborate rough and smooth endoplasmic reticulum, numerous ribosomes, and a prominent Golgi apparatus reflective of a high level of protein synthesis. A circumferential band of actin-containing microfilaments is located beneath the apical plasma membrane at the cell periphery and most likely is involved in maintaining cell shape and mediating cellular movement. Endothelial cells synthesize and secrete a thick basal lamina known as Descemet's membrane. Focal areas of increased electron density are present on the cytoplasmic aspect of the basal plasma membrane and may represent a form of adhesion plaque anchoring the endothelium to Descemet's membrane. Cytoplasmic processes extend from the basal aspect of the cells and penetrate Descemet's membrane, possibly contributing to increased adhesiveness of the monolayer.Focal tight junctions (maculae occludentes) on the apical aspect of the lateral membranes are small areas in which the outer leaflets of the plasma membranes of adjacent cells appear to fuse, obliterating the extracellular space. These junctional complexes do not form belts or rings extending around the cell, as are found in epithelia; rather, they occur as small zones of membrane fusion around the cell circumference. Electrical resistance across the endothelial monolayer is low (73 ± 6 ohm/cm2) compared with that across the corneal epithelium (1.6 to 9.1 kiloohm/cm2), reflecting the different organization of tight junctions in these two tissues. Gap junctions formed between adjacent cells are

located at all levels of the lateral plasma membrane below the tight junctional complexes. They possess a characteristic pentalaminar structure and are the site of electrical and metabolic coupling, which facilitates cell-to-cell communication.

PHYSIOLOGY

Barrier Function:

As an avascular tissue, the cornea receives oxygen mainly from the environment through the tear film, but its nutritional requirements are met by the aqueous humor. As such, the glucose, amino acids, and vitamins needed by the epithelial cells and stromal keratocytes must traverse the corneal endothelial monolayer. This nutrient transport occurs primarily through a paracellular route;

that is, solutes move between the cells rather than being actively transported through them. This form of transport requires that the endothelial monolayer be leaky to substances within the aqueous humor but not permit bulk fluid flow into the corneal stroma. The barrier to bulk fluid flow from the aqueous humor to the stroma is formed primarily by the focal tight junctions of the endothelium. Experiments with molecular tracers indicate that small molecules do not penetrate the tight junctions but rather enter the intercellular spaces by leaking around them.Gap junctions and the sinuous, elaborate interdigitation of the lateral plasma membranes together may form a secondary barrier to fluid flow. Gap junctions narrow the width between apposing cell membranes from the normal intercellular gap of 25 to 40 nm to about 3 nm.Narrowed intercellular spaces produced by the formation of gap junctions, in addition to the requirement that fluid must move between the sinuous interdigitating lateral membranes, help prevent bulk fluid flow across the endothelial monolayer.[7]

Pump Function:

Transparency is essential for the function of the cornea as the primary lens of the eye. Transparency results from the uniformity of the tissue elements comprising the cornea and from the regularity of their spatial organization. Precise arrangement of the collagen bundles within the corneal stroma is especially

important for corneal clarity.This precise arrangement depends to a great extent on the maintenance of a relatively low level of stromal hydration. Proteoglycans associated with the collagen fibrils within the stroma bind water, producing a natural pressure gradient across the endothelial monolayer. In addition, loss of integrity of the endothelial cell layer can hydrate the stroma. The disorganization of collagen fibrils that results from stromal swelling causes light absorbance, corneal clouding, and reduced vision.

The endothelium maintains a low level of stromal hydration by the activity of ionic pumps. Studies suggest that metabolic energy is needed to maintain normal corneal thickness. This energy requirement appears to be associated with the activity of specific adenosine triphosphatases (ATPases), located in the lateral plasma membrane, that catalyze ion exchange. These ATPases, which act as the metabolic pump, are believed to function by creating a net ionic flux from the intracellular to the extracellular milieu. The osmotic gradient produced causes water to be drawn passively from the stroma to the aqueous humor. At least two active transport systems appear to contribute to the pumping mechanism: an Na+,K+-ATPase pump and a bicarbonate-dependent Mg2+-ATPase pump.

The requirement that the endothelium permit passage of nutrients into the cornea and, at the same time, maintain a barrier to the free flow of water into the

stroma presents an interesting cell biologic paradox. The pump-leak hypothesis attempts to resolve this paradox, stating that the rate of leakage of water and solutes into the corneal stroma is balanced by the rate of pumping of excess water from the stroma back to the aqueous. As long as the equilibrium suggested by this hypothesis is maintained, the corneal stroma remains relatively dehydrated, and corneal clarity is maintained. Figure 56–14 illustrates this equilibrium. Any imbalance between the rate of fluid leak into the cornea and the rate of ionic pumping of fluid out of the cornea results in corneal swelling and loss of visual acuity.

Cell Division and Monolayer Repair

Corneal endothelial cells are capable of normal division during fetal development; however, the total corneal endothelial cell reserve is limited because cell division in adult cells is limited.At birth, endothelial cell density is 3500 to 4000 cells/mm2; in adults, this density is reduced to 1400 to 2500 cells/mm2.Cell density begins to decrease during fetal development as a result of both a rapid growth in corneal size and the limited mitotic activity that occurs after the second trimester of pregnancy. Once rapid corneal growth subsides, cell density continues to decrease, but at a slower rate. Beginning at about the second year of life,

decreased cell density is directly related to endothelial cell loss and the inability of the endothelium to reproduce in numbers sufficient to keep pace with this loss.The overall rate of cell loss accelerates if the endothelium is injured from trauma, disease, or dystrophy.[1,8]

Polymegathism (i.e., heterogeneity in cell size) increases in the endothelium with age and as the result of damage caused by trauma, corneal infection, or disease. Cell size can become heterogeneous for several reasons. When the endothelium is injured or when cells are lost because of normal attrition, repair of the defect in the monolayer occurs mainly through enlargement and spreading of neighboring cells, causing cells to be larger in these areas.In addition, the number of multinucleated cells and cells with greater than the normal diploid DNA content increase with age, producing a population of very large cells. Increased heterogeneity in cell shape (i.e., pleomorphism) also occurs with age or trauma.As the number of cells within the monolayer decreases and the cells enlarge, there is a decrease in the percentage of hexagonal cells within the monolayer. As polymegathism and pleomorphism increase, the endothelial monolayer can become destabilized. It is well known that a regular hexagonal pattern provides the greatest cellular packing with an optimal cell-to-membrane ratio. Irregular cell sizes and shapes can increase surface tension within the monolayer, producing decreased geometric and architectural stability.

When cell numbers are reduced as the result of aging or trauma and the remaining cells become larger and more pleomorphic, the ability to maintain or restore normal barrier and pump function can be compromised.With decreased monolayer stability, permeability increases, and the cornea can swell. Decompensation (i.e., loss of monolayer integrity and function) can occur when cell density falls below 300 to 400 cells/mm2 or when the mean cell size reaches about 3000 to 3500 µm2. Because of the stressed state of the endothelial monolayer under these conditions, the leak rate of fluid into the stroma becomes greater than the pump rate of fluid flow out of the stroma, producing stromal edema and corneal clouding. Transplantation is the normal recourse for reestablishing corneal clarity and visual acuity after decompensation of the corneal endothelium.

The endothelial cells form a uniform paving-stone mosaic of closely apposed polygonal cells with five to seven sides. These cells are about 20 microns in diameter, with a 250 square micrometer surface area. The endothelial population is uniformly distributed over the cornea and is symmetric in both eyes. In the periphery, the cells become progressively more irregular as they merge with the trabecular endothelium. At birth, the cell density is 4000-5000 cells/square mm arranged in a continuous monolayer 4-6 microns thick. As the endothelial cells age, they die. The adult human endothelial cells have a limited capacity to divide

and replace aging cells. But they enlarge\, re-organize themselves, migrate and maintain tight junctions.

The variables that are measured in endothelial cells are the cell size interms of density, spread of the cell size in terms of standard deviation or the coefficient of variation and the coefficient of skewness which measures the asymmetry of the cell population. Cells surrounding an endothelial defect enlarge to fill the defect and restore the monolayer. Hence, mean size of the endothelial cell tends to be greater in areas of endothelial trauma. Between the ages of 5 and 80 years the cell densirty ranges between 4000 and 900 cells/mm2.

Younger endothelial cells have a greater wound repair capacity and a greater functional reserve capacity to recover from an adverse event. Polymegathism describes heterogeneity in cell size, whereas pleomorphism denotes heterogeneity in cell shape. Both increase with age, trauma and disease. Irregular cell shapes produce an increased cell surface tension and decreased geometric stability. Thus, the less homogenous and hexagon-shaped the cell population becomes, the lower the capacity of the cells to withstand trauma.[8]

ENDOTHELIAL CELL ANALYSIS

Endothelial cell analysis provides important clinical information on corneal function and viability. The determination of the endothelial cell density (ECD) has become an accepted practice both clinically and in research to provide information on the cell layer needed to maintain corneal transparency .The potential clinical uses include the assessment of the endothelium in donor corneas, the monitoring of different anterior segment surgery techniques, and the longitudinal effects of intraocular surgery, such as cataract surgery or implantation of phakic intraocular lenses. When performing intraocular procedures, endothelial trauma should be minimized, and specular endothelial microscopy is recognized as being essential in evaluating the safety of new intraocular or corneal surgical procedures and intraocular lenses.[8]

Many studies have been published on the relationship of endothelial cell density and morphology with age, gender, and ethnicity. Although investigators differ in their findings about the relationship of age and gender to endothelial characteristics, it is clear that significant differences in corneal endothelial properties do exist among races and ethnic groups.

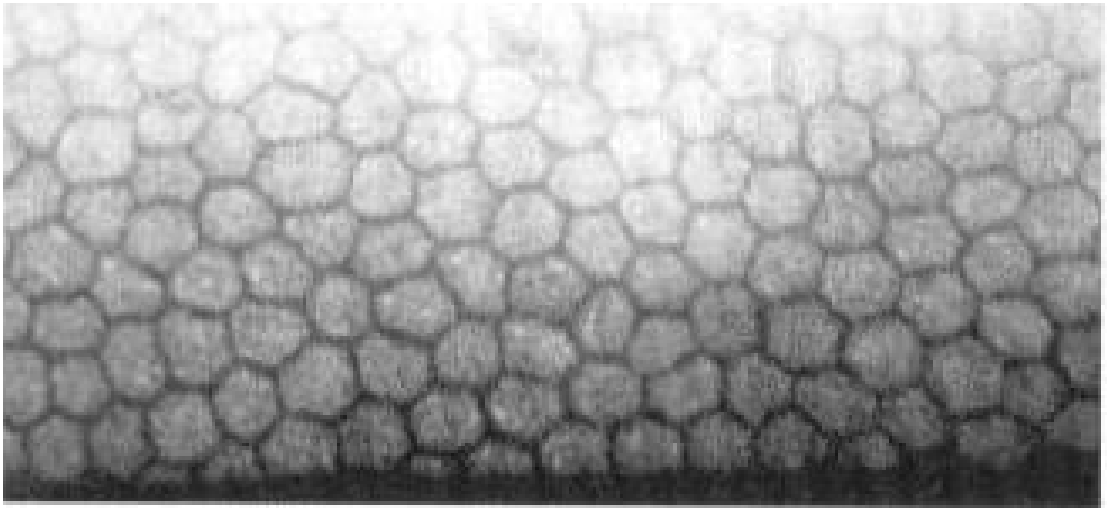
Noncontact endothelial imaging reduces the risk of corneal epithelial damage, artifacts due to corneal manipulation, and transmission of infection. The

disadvantage of this method is less control over patient eye movement, and therefore less resolution and magnification.A sample size of 50 cells may be adequate to study a normal cornea that does not have excessive pleomorphism. Many studies have compared contact and non-contact specular microscopy and have shown that contact specular microscopy is accurate and reproducible in the determination of endothelial cell density. The mean endothelial cell density in this study was within the range described for normal corneas.The cell loss rate with age (0.6% per year) was more than that described in most previous studies (0.3–0.5% per year), even as in longitudinal studies in which some subjects are examined again at a later date, a higher annual loss rate was reported (0.3–1.1% per year).

Endothelial cell loss also occurs in situations like trauma, surgery, contact lenswear and diabetes mellitus. In penetrating trauma including surgery where the anterior chamber is entered and manipulations are done within the anterior chamber, there is a possibility of direct injury to the endothelium. But in situationslike refractive surgeries and deep anterior lamellar keratoplasty, where the manipulaton is only on the surface of the cornea, endothelial cell loss is not expected.

The corneal specular microscope is a reflected-light microscope that projects light onto the cornea and images the light reflected from an optical interface of the corneal tissue, most typically the interface between the corneal endothelium and the aqueous humor. Depending on the instrument used, the projected light can be in the form of a stationary slit, a moving slit, or a moving spot and the optical design can either be non-confocal or confocal. Although specular microscopes have been used primarily to evaluate the corneal endothelium, the corneal epithelium and stroma as well as the crystalline lens can also be visualized and evaluated.

Normal corneal endothelium as photographed by specular microscopy. A quasi-



regular array of hexagonal cells all having nearly the same area is seen.

The young normal corneal endothelium above as seen by specular microscopy, shows a quasi-regular array of hexagonal cells all having nearly the

same size. With aging, trauma, and corneal disease, this regularity is lost. The goal of endothelial specular micrsocopy is to enable the status of the endothelium to be obtained by visual observation and morphometric analysis of the endothelial image. In general, the more the endothelial image varies from the normal appearance shown in the above figure, the more comporomized the endothelium, and the less able is the endothelium to provide its necessary functions that maintain corneal clarity.

Although observation of the corneal endothelium by specular reflection dates back to the early part of this century and observation of excized, non-moveable, rabbit orneas was accomplished it was not until a suitable instrument was developed by Laing in that clinical specular microscopy became a viable method. This original clinical specular microscope gave clinical photomicrographs having sufficient resolution to demonstrate individual cell boundaries and to distinguish numerous intracellular structures. From this early instrument the technique of clinical specular microscopy, as we know it today, was launched. Over the past 20 years there has been a continual improvement in the available technology that has resulted in instruments that are easy to use and that give superior image quality as compared to the early instruments.

Optical properties of a Specular Microscope[10]

To properly interpret the endothelial photomicrographs obtained clinically, it is helpful to understand the optical principles of the specular microscope. Light striking a surface can be reflected, transmitted, or absorbed. Generally, some combination of the three effects occur, with the relative proportions depending on such conditions as the wavelength of the light, the relative transparency of the medium below its surface, and the relative refractive indices on each side of the surface. Of primary importance in clinical specular microscopy is the light that is reflected specularly (i.e. “mirror-like”) where the angle of reflection is equal to the angle of incidence.

For the normal transparent cornea, most visible light incident on the epithelial surface is transmitted. As the light passes through the corneal tissue, some of it can be absorbed by the tissue and some can be reflected by nerve fibers, keratocytes, and other refractile objects (i.e. objects having a different index of refraction than the bulk corneal tissue). In the stroma of the normal cornea, most of the incident light is transmitted through the tissue, although a small amount is absorbed and/or scattered (reflected through arbitrary angles) by cellular organelles. With an increase in corneal edema the fraction of scattered light increases and can become the dominant element thus giving rise to a “hazy”

cornea. As light strikes the posterior corneal surface, almost all of it is transmitted into the aqueous humor. Because there is a change in index of refraction at the endothelium-aqueous humor interface, about 0.022 per cent of the total incident light is reflected; this reflected light is captured by the clinical specular microscope and forms the endothelial image.

As the illumination beam of the specular microscope passes through the cornea, it encounters a series of interfaces between optically distinct regions. At each of these interfaces, some light is reflected back toward the photomicroscope and some is transmitted deeper into the cornea. The greater the difference in index of refraction between the two regions, the greater the amount (intensity) of the reflected light.The more edematous the tissue, the greater the intensity of scattered light. A portion of the reflected and backscattered light is collected by the objective lens of the specular microscope and forms, at the image plane of the microscope, an image of that part of the cornea on which the instrument is focussed.

A narrow slit of light from a specular microscope is focused onto the posterior corneal surface. The incident light illuminates, in turn, the precorneal tear film or a coupling medium (e.g., artificial tears, methyl cellulose, etc.) between the objective lens and the cornea, the epithelium, Bowman's membrane, the stroma, Descemet's membrane, the endothelium and the aqueous humor. Within each of

these regions, in the absence of corneal edema, only a small amount of light is scattered back towards the image plane, while at the major optical interfaces (labeled 1,2,and 3) much more light is specularly reflected back toward the image plane. Using indices of refraction for the objective lens, saline, cornea, and aqueous humor of 1.517, 1,333, 1,376, and 1.336, respectively, the fraction of light reflected from each of these interfaces can be calculated to be 0,36% from the objective lens-saline interface, 0.025% from the saline-corneal epithelium interface, and 0.022% from the corneal endothelium-aqueous humor interface. Intracorneal optical interfaces (e.g., between epithelium and Bowman's membrane or between stroma and Descemet's membrane, etc) also reflect light, but the fraction of reflected light cannot be calculated because the index of refraction of the separate layers of the cornea has never been measured.[11,12]

At the image plane of the specular microscope, light from various corneal regions and interfaces overlaps. Whenever a bright region and a dark region overlap, the dark region is not seen. If a sufficiently narrow slit of incident light is used, one can generally distinguish a bright region (Zone 1), part of the stromal region (Zone 2), the endothelial region (Zone 3), and part of the aqueous humor (Zone 4). Zone 1 is formed by light reflected from the lens-coupling fluid or the coupling fluid-epithelial interfaces or both, depending on the index of refraction of the coupling fluid used.[13, 14, 15]

The demarcation line between Zone 3 and Zone 4 that separates the illuminated cornea from the nonilluminated structures located more posteriorly, is called the dark boundary. One side of the boundary is dark because negligible light is scattered from the aqueous humor. In contrast, the demarkation line between Zone 2 and Zone 3 is called the bright boundary. This boundary separates the endothelial reflection from the overlying illuminated corneal stroma. Since some light is scattered from the stroma, neither side of the boundary is dark.

. In scanning slit and scanning spot confocal specular microscopes, the slit width (or spot diameter) is made very small to give, at least in theory, an increased image quality and the large field desired is accomplished by moving the illuminated slit (or spot) over the endothelium, stromal nerves, keratocytes, etc.[13, 16, 17]

Using a narrow slit four distinct zones are seen; with a wide slit only three zones are apparent. A bright zone, Zone 1, arises from the reflection at the objective lens-epithelial cell interface. Zone 2 arises from light diffusely scattered from corneal stroma and is present only in narrow slit photographs. This zone is darker in clear corneas and brighter in edematous corneas. Zone 3 shows the endothelial cell pattern produced by light specularly reflected from the posterior corneal surface, while Zone 4, the dark zone, is the product of light scattered from

the aqueous humor. Since little light is scattered in the aqueous humor and virtually no light from the region normally returns to the collection optics of the microscope, Zone 4 is generally dark. In eyes with considerable debris in the anterior chamber this zone occasionally can be brighter and show some structure, but in most instances it is uniformly dark.[18, 19]

Qualitative Morphometric Analysis of Specular Images

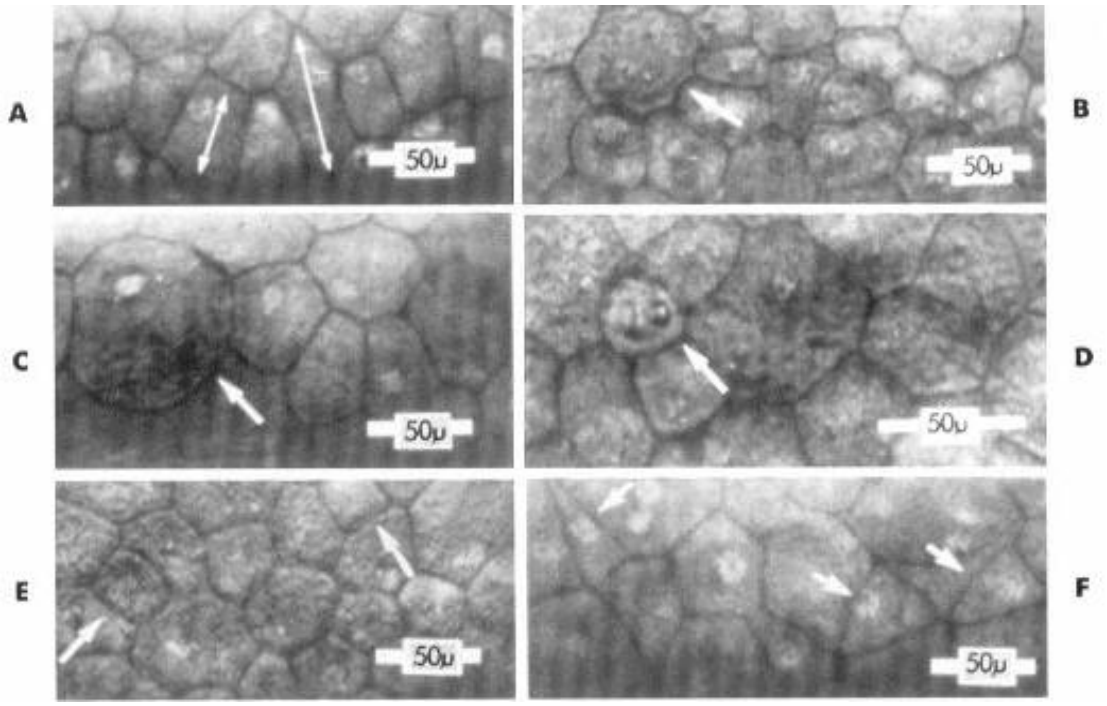
Both qualitative and quantitative assessments of the corneal endothelium can be made. Qualitative cellular analysis identifies abnormal endothelial structures and grades the endothelium either according to the number or size of the abnormal structures present or on the basis of an overall visual assessment of endothelial appearance. The goal is to provide a subjective evaluation of the endothelium, not to assign a precise numerical value to the specular photomicrograph. This type of analysis provides a rapid clinical evaluation of the endothelium to assess the risks of intraocular surgery, to establish a diagnosis, or to decide upon treatment. Complete qualitative analysis requires that several parameters be evaluated including cell conformation, cell boundaries and their intersections, configuration of the dark boundary, and the presence of acellular structures. One must be careful to eliminate optical artifacts from consideration when performing either qualitative or quantitative analysis of the corneal endothelium.

Cell Conformation[20, 21, 22]

The specular microscope shows A pattern of contiguous cells having well defined cell boundaries. The central endothelial cells of young people with normal eyes are hexagonal and approximately the same size; the distribution of cell area is approximately Normal (Gaussian). With age, the average cell area increases, the

cellular pattern becomes distinctly pleomorphic, and the cell size distribution becomes skewed toward larger cell areas. In young people with normal eyes the cell side lengths are all roughly equal. In older individuals, the side lengths lose this regularity and one sees an increasing variation.

Variations in the configuration of the corneal endothelium. A, Elongated cells. B, Cell

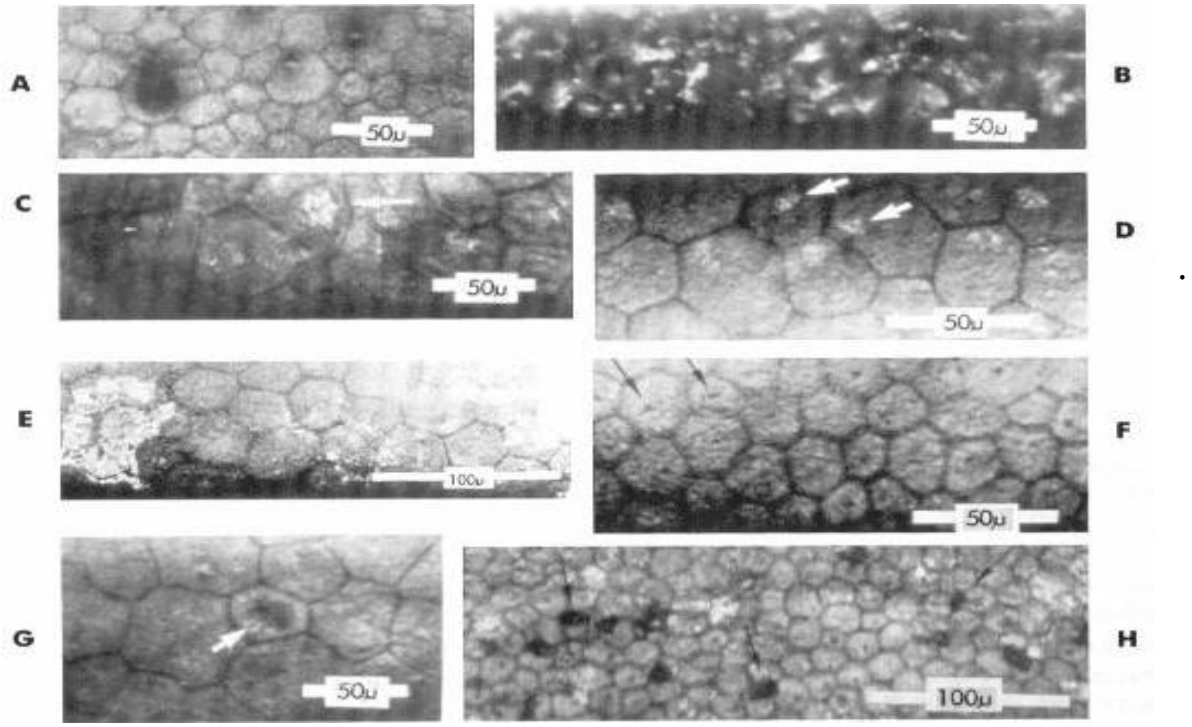


having scalloped edges. C and D, Round cells. E, Square cell. F, Triangular cell.

In addition to changes in size, endothelial cells may assume a shape that is substantially different from the usual quasi-hexagonal configuration. Figure A shows endothelial cells that are both enlarged and elongated. These cells were encountered near the corneal apex in a case of keratoconus. They appear to be aligned in the same direction and to follow lines of stress as if they have been

stretched by gross deformation of the cornea. Cells with scalloped rather than conventional straight sides are occasionally seen (Fig. B). So, too, are round cells (Fig. C and D), square cells (Fig. E), and triangular cells (Fig. F). As in the case with changes in endothelial cell size, alterations in shape have not been directly related to changes in the physiologic function of the affected cells.

Miscellaneous Structures



A number of inter- and intra-endothelial cell structures, which may be either dark or bright in appearance, are seen in endothelial photographs. One type of dark structure disrupts the endothelial cell pattern and can range in size from a structure smaller than a single cell to one larger than an individual endothelial cell. Each such structure generally has dark sides and a central bright spot (Fig. A). Such

structures represent a smooth excrescence of Descemet's membrane (i.e., cornea guttatae) and often are surrounded by a ring of abnormally shaped cells. Cornea guttate can be seen at a much earlier stage with the specular microscope than with the conventional slit-lamp biomicroscope. When these excrescences are abundant, they begin to touch one another and to coalesce. One sees this pattern illustrated in Fig. B; although the endothelial cell pattern is not visible, the bright reflection from the apex of each excrescence is clearly seen.

Two additional types of dark bodies, both intracellular in location, have been observed. The first type is small, generally located in the central or paracentral portion of the cell, and has sharp, well-defined edges showing that it is located on the posterior surface of the cell (Fig. F). This structure has been seen in clinically normal corneas, and when present, it occurs in many but not all cells. It presumably represents the base of an endothelial cilia, although histological verification of this has not yet been obtained. The second type of dark body is considerably larger and has indistinct edges, suggesting that it is located within the cell (Fig. G). It may represent an intracellular vacuole or bleb.

Intercellular dark structures, lying predominantly at endothelial cell intersections, have been observed (Fig. H).They tend to be uniform in size, and within a given frame they are randomly positioned across the endothelial cell

pattern. These structures occur in patients with anterior uveitis, and it is believed they represent invading inflammatory cells.

Intracellular bright structures, some of which may be only the cell nucleus, have been seen in endothelial photomicrographs.They are variable in size and typically are contained completely within a single endothelial cell (Fig. C and D).

Occasional exceptions do occur, however, where the bright structure seems to cross a cell boundary. These intracellular bright structures appear to be associated with stressed cells, explaining why they commonly are seen within enlarged cells such as those encountered in successful corneal transplants. Their size or number (multiple bright structures may occur within a single greatly enlarged cell) seems to be proportional to the size of the cell. That is, the larger the endothelial cell, the larger the intracellular bright structure.

A second type of bright structure spans several endothelial cells (Fig. E) and is positioned at random on the endothelial cell pattern of the specular photomicrographs. When viewed directly, these structures appear to sparkle; some are orange, while others are white. Slit-lamp biomicroscopy of these corneas reveals numerous pigment deposits on the endothelium. The bright structures seen with the clinical specular photomicroscope appear to correspond to the pigmented

endothelial deposits seen with the slit-lamp biomicroscope, and presumably they are the same abnormality.

Morphologic variations in endothelial cell configuration, cell surface properties, and intercellular boundaries, as well as the presence of numerous intracellular structures, can be identified by the clinical specular microscope. Although the nature and significance of many of these abnormalities are not presently known, their recognition represents an initial step in the elucidation of their pathophysiological significance.

Quantitative Morphometric Analysis of Specular Images

Although a qualitative description of the corneal specular image suffices for many applications, more quantitative information is desirable for others. The aim of quantitative analysis is to assign a number (or set of numbers) to the specular photomicrograph that can provide a measure of the endothelial status.

Various of morphological parameters that can be quantified. These include cell size (cell area or cell density), polymegathism (variation of cell size such as coefficient of variation of mean cell area), pleomorphism (variation of cell shape such as percent of hexagonal cells or coefficient of variation of cell shape), cell perimeter, average cell side length, cell shape, and so forth. Histograms or

frequency distributions of these quantities can also be determined. To date only cell size, pleomorphism, and polymegathism and several variables related to these parameters have proven useful in determining endothelial status.[23, 24]

Two equivalent parameters have been used to quantify endothelial cell size. They are mean cell area and cell density (or cell count). Cell area has most often been expressed in units of μm^2 per cell and cell density in units of cells per mm^2. The two quantities are related by the following equations:

Mean cell area (μm^2/cell) = 10E6/cell density (cells/mm^2)

or

Cell density (cells/mm^2 = 10E6/mean cell area (μm^2/cell)

Two different methods, fixed frame analysis and variable frame analysis, can be used to measure either of these two parameters of cell size.

Fixed Frame Analysis of Cell Size

In fixed frame analysis one counts the number of cells within a frame or window of constant area. All cells lying completely within the frame are counted as whole cells. However, along the boundary of the frame there are many cells that lie only partly in the frame, and for these cells it is usually impossible to determine

the fraction of the cellular area that lies within the borders of the frame. Each cell that is only partially within the frame is counted as one half cell regardless of the fractional area of that cell located within the frame. The total number of cells (the cell count) is then taken as the sum of the number of whole and half cells within the frame. To speed up the counting process one commonly invokes a symmetry principle and counts all cells cut by two sides of the frame as whole cells and does not count those cells cut by the other two sides of the frame. As long as the number of boundary cells is small compared to the total number of whole cells within the frame, and cellular pleomorphism is not too great, this method can give reasonably accurate values for mean cell size. The size is obtained by dividing the cell count by the area of the frame and expressed as cell density in cells per mm^2. The area of the frame must be referred to the endothelium. This is accomplished by dividing the actual area of the frame by the square of the linear magnification of the specular microscope, and if the cells were counted from an enlargement of the negative, by the square of the linear magnification of the enlargement. One can also divide the area by the cell count and report mean cell area as well. In practice, except for very rough estimates of cell count, a minimum of 35 contiguous cells should lie within the counting frame, although for most studies it is preferable to have 50 to 100 cells within the counting frame. Otherwise the errors associated

with the counting method itself will generally be too large to provide meaningful numbers.[25]

Variable Frame Analysis of Cell Size

Variable frame analysis, originally proposed by Laing, is most conveniently done using a computer based analysis system such as the Bambi system (Bio-Optics, Inc. Arlington, MA). This method eliminates the problem of counting fractional cells along the boundary, thus providing a more accurate determination of mean cell size than fixed frame analysis, again assuming that cellular pleomorphism is not too great and that the cell sample is representative of the area under study.In variable frame analysis, one first measures the variable area occupied by an integral number of cells by tracing around a contiguous group of cells with a mouse. The user then marks each cell by clicking it with the mouse. The computer then calculates the cell density by dividing the number of marked cells by the area of the frame. An equivalent value, the mean cell area, can also be obtained by dividing the frame area by the number of cells that have been circumscribed.

Using variable frame analysis, errors due to the counting method are very small as compared to fixed frame analysis so that many fewer cells must lie within the frame. Furthermore, with variable frame analysis, additional information can be

obtained. If one traces around the smallest cell in the image and marks it, the computer calculates the cell density assuming that all cells are this small. If one then traces around the largest cell in the image and marks it, the computer calculates the cell density assuming that all cells are this large. These two numbers give the range of cell densities (or the range of cell areas) that exist in this image providing more information regarding the endothelium than if only the mean cell density is obtained.[26, 27]

Individual Cell Analysis

In fixed frame analysis only average cell size can be determined. The same holds true for variable frame analysis if only a group of cells is circumscribed. However, using the variable frame technique, single cells can be traced with the stylus of the planimeter or digitizer, and this then permits individual cell analysis. Such an analysis provides much more information about the endothelial cell pattern than can be obtained with methods that determine only cell density or average cell area.

Individual cell analysis can be performed either manually, semi-automatically, or fully automatically. The first computerized manual analysis system was developed by Laing and associates. In this system (the Bio-Optics Mandig system) the cell boundaries are traced with a special pen or a cross-hair

cursor of a digitizer. As this is being done, the x and y coordinates of the boundary points are automatically entered into a computer. The computer determines when the cell has been completely circumscribed and calculates the area (or other programmed morphologic parameters) of that cell. It then instructs the operator to trace another cell. The cell density or mean cell area can be obtained by averaging the data on a group of cells. In addition, a frequency distribution (or histogram) of cell size can be obtained. Although such frequency distributions provide considerable information about the endothelium, few studies were done before the use of the personal computer since the procedure was extremely tedious.

Laing et al[10] developed semi-automated methods utilizing video images that eliminated much of the tedium associated with endothelial analysis. These methods also enabled additional morphometric parameters to be easily determined. The system developed (the Bio-Optics Bambi system) requires the initial digitization of the cells using a mouse and then calculates all of the morphometric parameters believed to be possibly important. The system enables the addition of new programs to calculate additional parameters should this be desirable. Poor endothelial images can be contrast-enhanced to improve them. Images and data files can be instantly printed, stored, retrieved, and transferred to other computers, as desired. This system is widely used for the evaluation of the endothelium.

Fully automated methods of individual cell analysis in which the computer accomplishes the task of determining cell borders and cell apices from background noise in the image as well as performing the morphometric and statistical calculations have been under development for many years. Such fully automated systems have been developed by Laing and associates, by Nishi and associates by Hartmann and associates (which system was improved by Fabian and associates, by Assenbauer and associates, and by Corkidi and associates and by several companies that, so far, have not published the methods or computer algorithms used. Due largely to the complex nature of the endothelial image, (especially for the abnormal endothelium) all of the systems so far developed make a variety of errors and none have been able to obtain reliable numbers without tedious and time-consuming manual editing ofthe image and/or graphic cell borders calculated and displayed by the computer. Because of the complexity of an endothelial photograph, fully automated methods, at least for the foreseeable future, will require considerable interaction and decision-making by a human operator. [29, 30]

Endothelial wound healing mechanisms: sloughing & sliding, coalescence, and mitosis[3, 6, 23]

Prior to the development of clinical specular microscopy it was believed that the only healing mechanism for the adult human corneal endothelium was the

"sloughing and sliding" mechanism whereby damaged endothelial cells sloughed from the endothelium and the adjacent undamaged, or less damaged, cells moved laterally so as to cover the defect left by the sloughed cells. Specular microscopy revealed two additional healing mechanisms. Endothelial cell coalescence (cell fusion), is a mechanism in which the common cell membrane between two cells degenerates to result in a larger cell containing two nuclei and, presumably, all of the cellular organelles of the two individual cells. Endothelial cell mitosis, once generally believed to be impossible for adult human endothelial cells, has also been demonstrated in the adult human cornea following successful treatment for graft rejection. Although considerable effort has been expended to induce mitosis by the application of exogenous substances such as growth factors, such efforts have been largely unsuccessful and the trigger for endothelial cell mitosis has been elusive.

LAMELLAR KERATOPLASTY

Arthur von Hippel performed the first LKP in the last quarter of the 19th century. The basic principle is to replace only that part of the cornea that is diseased and leave the recipient’s normal corneal layers intact. The idea is to to do the least amount of resection with the greatest amount of benefit thus leaving the healthy endothelium and Descemet’s membrane as an immunological barrier to rejection[3, 4, 6]

Types

Inlay LK: A part of the anterior stromal lamellae of the recipient is removed and replaced with healthy partial thickness donor cornea, consisting o stroma, Bowman's layer and epithelium. It is used to treat superficial corneal scars

Onlay LK

A partial thickness donor cornea is placed on a de-epithelialised cornea in which a small peripheral keratectomy and/or peripheral lamellar dissection has ben done. Epikeratoplasty done for keratoconus and keratoglobus is an example of onlay LK

Anterior LK

Encompasses both inlay and onlay LK

Deep LK

Deep resection or ablation of host stroma upto the level of the Descemet's membrane and replacement with donor stromal button. It is performed for keratoconus and corneal scars.

Posterior LK:

The anterior layers of host cornea are preserved and the posterior layers are replaced.

Indications

Optical
Therapeutic
Tectonic

Optical

Superficial corneal scars
Irregular/ecstatic corneas

Tectonic

Peripheral corneal thinning

Ectatic pathologies

Therapeutic

Recurrent pterygium
Conjunctival CIN

Major indications

Corneal dystrophies
Aniridia keratopathy
Corneal scars
Keratoconus

In developing countries

Chemical injuries
Trachomatous keratopathies
Dermoids

Criteria for LK

Reasonably healthy host ocular surface
Optimum endothelial function
Corneal opacity that spares the Descemet's membrane
Grossly distorted cornea that precludes Contact lens fitting

Contraindications

Herpes simplex/zoster
Chemical or radiation injuries
Stevens-Johnsons syndrome
Neurotrophic keratopathy
Lagophthalmos with exposure keratopathy
Severe dry eye

DEEP ANTERIOR LAMELLAR KERATOPLASTY

Deep anterior lamellar keratoplasty (DALK) is a surgical procedure for removing the corneal stroma down to Descemet's membrane. It is most useful for the treatment of corneal disease in the setting of a normally functioning endothelium. Traditionally, penetrating keratoplasty (PK), which involves a full-thickness corneal graft, has been the treatment of choice for corneal stromal diseases. But PK can be complicated by graft rejection, irregular astigmatism and

corneal opacification, thus resulting in visual impairment. DALK offers an alternative procedure that may lessen those risks because the recipient Descemet's membrane and endothelium are preserved. At the same time, DALK carries the potential danger of decreased visual acuity due to possible opacification at the interface layers.

As early as the 1950s, Jose Barraquer and colleagues in Colombia applied new techniques of lamellar keratoplasty, dissecting the corneal stroma down to two-thirds of its thickness in both the donor and the recipient tissue. Yet the procedure failed to gain favor because of poor visual outcomes related to irregularity of the dissected surfaces and scarring in the tissue interfaces. Although exposure of Descemet's membrane in corneal dissection was performed in the 1970s, the term "deep lamellar keratoplasty," as it is used today, was not employed until 1984 by Eduardo Arenas Archila, MD, with the use of intrastromal air injection to facilitate host tissue removal. By the late 1990s, studies indicated that DALK was associated with visual outcomes similar to PK without the risk of immunological rejection. In spite of positive reports in the literature, the classic technique of layer-by-layer stromal tissue removal was tedious and required great surgical experience, thereby limiting its use around the world. Recent advances in techniques contributed by many surgeons have started to popularize the procedure.

Indications

DALK can be an effective treatment for any pathology of the anterior cornea (epithelium, Bowman's layer and stroma) as long as the patient has an intact, functioning endothelium. Common indications for DALK include keratoconus and corneal scars. Patients with keratoconus are good candidates for DALK because they are typically young and have healthy endothelium. These patients stand to lose the most from the occurrence of post-PK immunological reactions that can compromise endothelial function in up to 20 percent of cases. Less common indications for DALK include vernal keratoconjunctivitis, corneal dystrophies and ocular surface diseases with limbal stem cell deficiency, including Stevens-Johnson syndrome, ocular cicatricial pemphigoid and chemical/thermal burns.

Pros and Cons

DALK offers a variety of theoretical and practical advantages compared with PK for patients with a healthy corneal endothelium.

Pros: Anatomic outcomes

Eighteen percent of primary full-thickness grafts have been reported to fail within 10 years due to endothelial rejection and chronic endothelial cell loss, and DALK may reduce this rejection rate because the host endothelium is preserved. In

a randomized clinical trial comparing DALK and PK published in 2002, there was a significant difference in endothelial density between treatment groups at 24 months postoperatively (2,183 ± 300 vs. 1,868 ± 272 per mm^2 respectively, P = 0.044). Furthermore, while endothelial cell density stabilized around six months after DALK, there continued to be loss of cells in the PK group at 24 months. Moreover, keratometric astigmatism rates were lower at six and 12 months after DALK compared with the same intervals after PK. Additionally, studies indicate a shorter healing time (suture removal at four months after DALK vs. 12 months with PK), fewer postoperative complications and shorter use of topical steroid treatment with the deep anterior approach. Finally, from a public health perspective, DALK provides the opportunity to use donor tissue with questionable endothelium that might not be suitable for full-thickness grafts. In the future, it may be possible to use the same donor cornea for a DALK and a Descemet's stripping endothelial keratoplasty.

Visual outcomes

In spite of the clinical and theoretical advantages of lamellar keratoplasty, visual outcomes have not always been ideal. Visual acuities associated with older lamellar keratoplasty techniques were inferior to those associated with PK due to the tissue interface scarring that developed between the donor cornea and the

recipient stromal bed. However, since deep lamellar keratoplasty surgery removes almost all of the recipient stromal tissue, the risk of scarring in the interface and subsequent poor visual outcomes is much reduced.

Uncertain

Currently, the literature is ambivalent regarding visual outcomes between DALK and PK. In one comparative cohort study, the proportion of patients who achieved a BCVA of 20/20 was significantly higher following PK compared with DALK (70 percent vs. 22 percent, P = 0.04); however, in another randomized, controlled trial, there was no statistically significant difference between the two procedures in associated postoperative BCVA. Ultimately, the visual result of DALK depends on the ability to maintain clear interfaces between the tissues. The femtosecond laser has been successfully used in donor human eyes to cut posterior lamellar flaps in endothelial keratoplasty, and this technology could potentially reduce tissue irregularity and scarring in DALK.

Cons

The disadvantages of the procedure are the complexity and novelty of the techniques. A learning curve exists, and cases of recipient corneal perforation requiring transition to PK may require another donor eye with better endothelial quality.

Performing DALK[6, 7]

Various approaches for performing DALK have been described in the literature. One method is to remove the host anterior corneal tissues layer-by-layer until reaching the deep stroma or the bare Descemet's membrane. Although different surgical techniques vary in their details, the basic surgery consists of the following steps:

Recipient eye

The anterior corneal surface is cut with a suction trephine set to a depth of about two-thirds of the corneal thickness. Then the stromal layers are dissected with a rounded blade, angled parallel to Descemet's membrane. Fluid or air is then injected using either a 27- or 30-gauge cannula in between the deep stroma and Descemet's membrane to separate those layers. Because early techniques failed to visualize the depth of stromal dissection, there was a greater risk of perforation than when Descemet's membrane can be visualized. New techniques described below can decrease surgical times while improving the safety and success rates of DALK.

- **Intrastromal air injection.** One innovation in DALK has been the use of an air-filled tuberculin syringe, the needle of which is injected obliquely into the

stroma prior to trephination. This intrastromal air renders the cornea opaque and provides a safe deep interface for the trephine.

- **Hydrodelamination.** After the initial trephination to about 75 percent of the corneal thickness, the surgeon can inject balanced salt solution through a cannula into a small pocket created in the central stroma. The saline induces stromal fiber swelling, which facilitates fine manipulation with forceps.
- **Viscoelastic dissection.** After an initial 80 to 90 percent trephination, sodium hyaluoronate can be injected through a blunt cannula deep into the central corneal lamella near Descemet's membrane. Injection of the viscoelastic substance between the deep stroma and Descemet's membrane facilitates the separation of the final layers.
- **Big bubble.** With the "big bubble" technique, air is injected deep into a groove created by trephining 60 to 80 percent stromal thickness. This introduction of air into the stroma anterior to Descemet's membrane creates a dome-shaped detachment of Descemet's membrane, which is then identified by a ring visible with the microscope.
- **Anterior chamber air.** In order to obtain the best visualization during the surgery, Gerrit R. J. Melles, MD, PhD, suggested introduction of air in the anterior chamber. This injected air creates a mirror like effect that facilitates the movement of surgical instruments between Descemet's membrane and the deep

stroma. Furthermore, the air-to-endothelium interface becomes a landmark to identify the posterior surface of the cornea, serving as a reference for dissection depth.

Donor eye

Although preserved grafts can be used, most of the literature reports use of fresh corneas prepared by the surgeon. Descemet's membrane and endothelium are removed by gently swabbing the posterior corneal surface of the donor corneoscleral rims with dry cellulose sponges. Forceps also may be used for removing the posterior corneal layers. Then a corneal button is punched out from the tissue. Suturing technique (interrupted, running or combined interrupted-running 10.0 nylon sutures) can be done according to the surgeon's preference in DALK, just as in PK. After suturing, a bandage soft contact lens is placed on the cornea.

Complications

The most frequently encountered complication of DALK is perforation of Descemet's membrane and entering the anterior chamber from the stroma. Descemet's membrane and the posterior cornea are especially susceptible to perforation by sharp instruments. Tears or perforations occur in approximately 10 to 30 percent of cases.[2] The viscoelastic dissection technique may offer some

protection against perforation. If a perforation occurs, management depends on the timing and the size of the injury. If the tear occurs while the stroma still covers Descemet's membrane, it generally self-seals. Small perforations occurring during dissection may result in postoperative Descemet's membrane detachment. Most Descemet's membrane detachments can be managed by injecting air or an air/gas mixture (sulfur hexafluoride + air) into the anterior chamber after surgery or under the slit lamp postoperatively, thereby creating a tamponade to seal the Descemet's membrane defect. However, it should be noted that the use of a tamponade to reattach Descemet's membrane may lead to closure of the anterior chamber angle from pupillary block. A rare complication occurs when a fixed, dilated pupil ensues secondary to iris ischemia, referred to as the Urrets-Zavalia syndrome. In cases where the tamponade fails to manage the perforation, a conversion to traditional penetrating keratoplasty might be necessary.

Postoperative Care

Topical corticosteroids and antibiotics are administered postoperatively. Patients are monitored for inflammation, infection, graft rejection, astigmatism and suture-related problems. Overall, patients tend to fare well with a shorter course of corticosteroids after DALK than after PK. At 12 months postoperatively in one study, only 15 percent of DALK patients used topical prednisolone, while 75

percent of PK patients continued to need the treatment. In addition, sutures can be generally removed earlier in DALK patients than in PK patients.

With a long history, DALK is seeing a recent renewed interest. Although DALK has the reputation of being challenging and time-consuming, recent advances in techniques have cut the surgical time considerably. In patients with anterior corneal opacification or structural corneal defects with a healthy, functioning endothelium, DALK can be considered as the first-line treatment due to its preservation of host endothelium and Descemet's membrane. If future studies show that DALK provides lower postoperative complications, fewer graft rejections and similar visual outcomes compared to PK, DALK should have a promising future in corneal surgery as a viable alternative to full-thickness PK in selected patients.

DALK AND ENDOTHELIAL CELL LOSS

Though DALK has been touted to be a better procedure than PKP in the maintenance of the endothelial cell layer of the cornea, there have been studies which have proved otherwise. A study done by Patel et al at the Princess of Wales hospital, UK, showed that the endothelial cell loss was the same in two eyes of the same patient with macular dystrophy, one of which underwent a DALK and the other, PKP.

Further, a study done by van Dooren BT et al at the Rotterdam Eye hospital Netherlands has shown that there is a significant endothelial cell loss in patients who underwent DALK irrespective of the antecedent etiology. The endothelial cell loss was significantly higher than the physiological endothelial cell loss.[33, 34, 35]

AIMS OF THE STUDY

a) To determine the viability of the graft after Deep Anterior Lamellar Keratoplasty

b) To assess the visual acuity after Deep Anterior Lamellar Keratoplasty

c) To determine the loss of corneal endothelial cells after Deep Anterior Lamellar Keratoplasty

Inclusion Criteria

a) Lesions involving the anterior 2/3rd of the corneal stroma

b) No evidence of active inflammation

c) No evidence of vascularisation of the cornea

d) In keratoconus patients, those that could not be corrected with spectacles or contact lenses.

Exclusion Criteria

a) Lesions involving the full thickness of the cornea

b) Iris incarceration to the corneal lesion

c) Presence of active inflammation

d) Presence of vascularisation

MATERIALS AND METHODS

The study was conducted at the Cornea Services of the Regional Institute of Ophthalmology, Government Ophthalmic Hospital, Chennai between June 2007 and November 2009.

27 eyes of 27 patients who underwent Deep Anterior Lamellar Keratoplasty was included in the study.

These 27 patients underwent Deep Anterior Lamellar Keratoplasty for various etiologies.

Their pre-operative visual acuity, both uncorrected and best corrected, was assessed using a Snellen's chart.

A thorough ocular examination was done. The ocular adnexa was assessed for the presence of any active inflammation. The eyelids were assessed for the presence of any lid abnormalities such as entropion/ectropion, trichiasis or lagophthalmos. The intergrity of the Bell's phenomenon was assessed.The ocular surface was examined for the presence of inflammation. Tear film abnormalities were ruled out. Slit lamp biomicroscopy was done to assess the depth of the corneal lesion, to determine the presence of active inflammation and to rule out the presence of vascularisation of the cornea. The other ocular structures were examined for their integrity.

All patients included in the study underwent Deep Anterior Lamellar Keratoplasty by the same surgeon, using the technique of lamellar dissection.

After the patients underwent Deep anterior Lamellar Keratoplasty, their eyes were examined to assess the status of the graft, the uncorrected and best corrected visual acuity and the endothelial cell count.

The endothelial cell count was assessed using a SP 2000P specular microscope (Topcon).

The patients were followed up over a period of one year and at each visit a detailed ocular examination, an uncorrected and best corrected visual acuity and a specular microscopic examination was done.

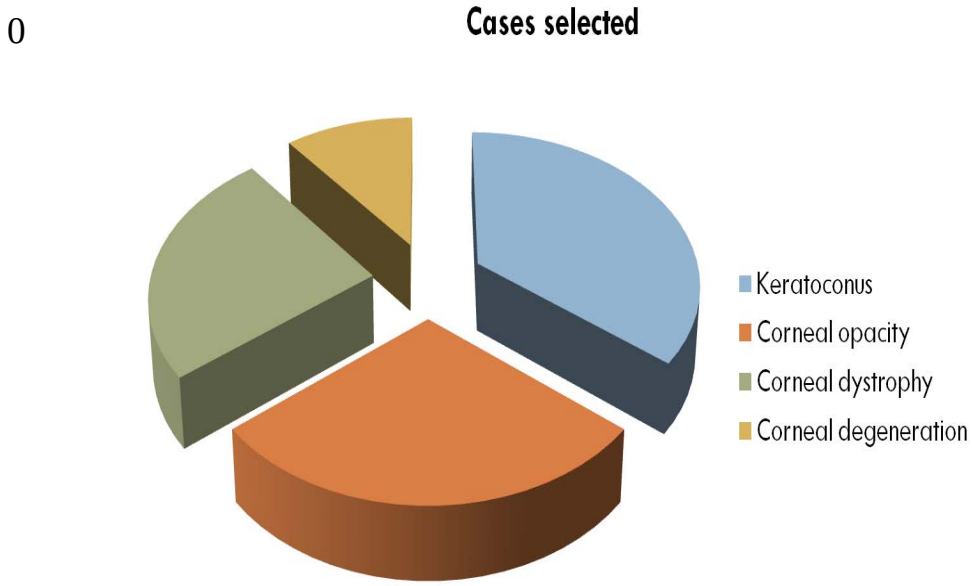
ANALYSIS AND RESULTS

The various etiologies for which deep anterior lamellar keratoplasty were performed

Table – 1

Etiology	Number of eyes	Percentage
Keratoconus	8	29.6
Corneal Dystrophy	6	22.2

Corneal Opacity	7	25.9
Corneal Degeneration	4	14.8
Limbal dermoid	1	3.7



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Analysis of graf **etiology**

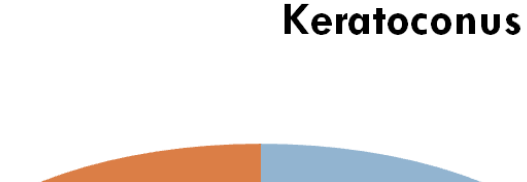
for which DALK was performed

1) KERATOCONUS

Status of graft

Table – 2

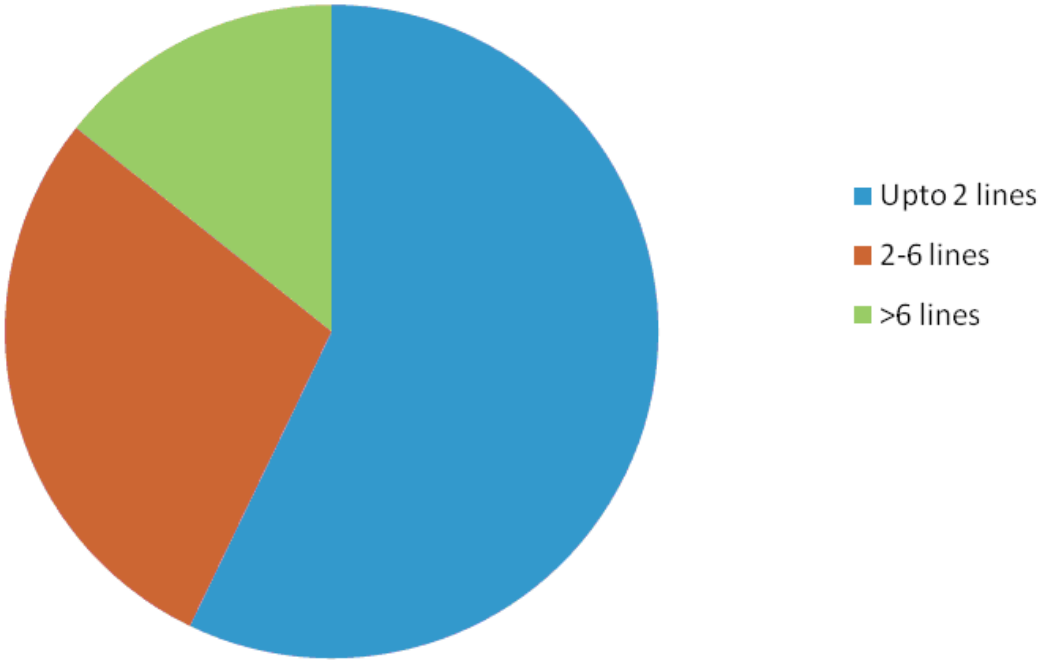
CLEAR	OPAQUE	PERCENTAGE
7	1	88%



Improvement In Visual Acuity

Table - 3

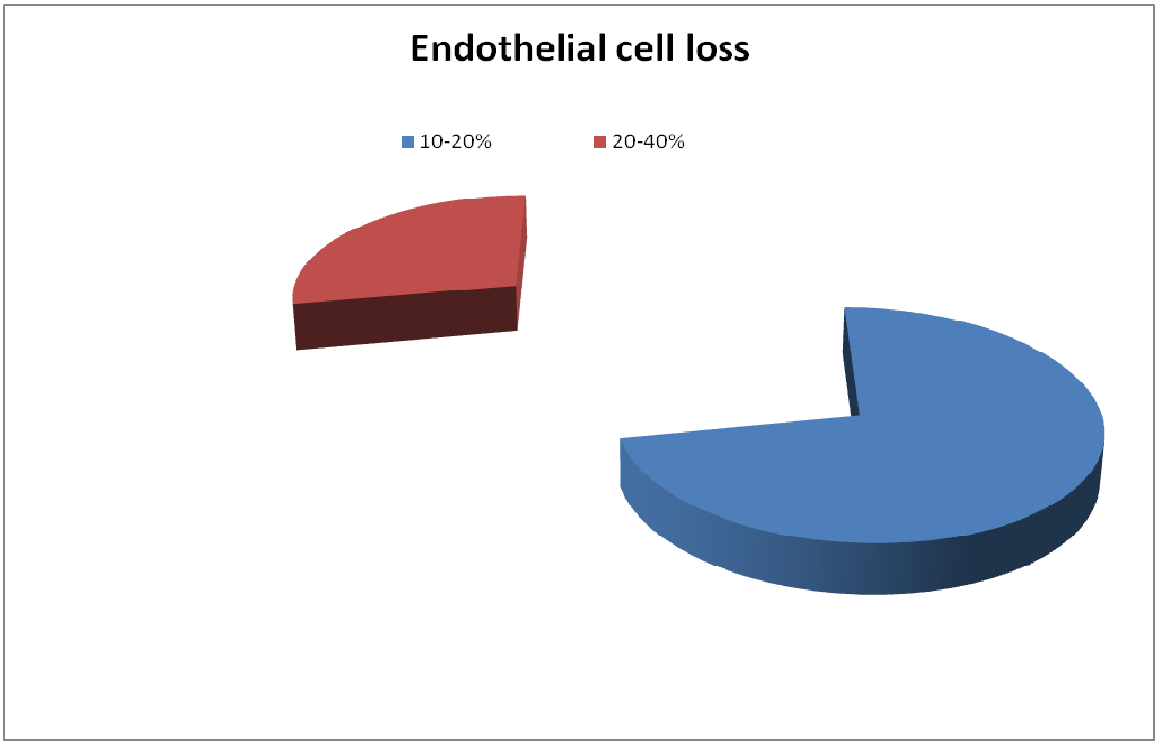
Upto 2 lines	2-6 lines	> 6 lines
4	2	1
57.1%	28.5%	14.2%



Endothelial cell loss

Table - 4

10-20%	20-40%
5	2



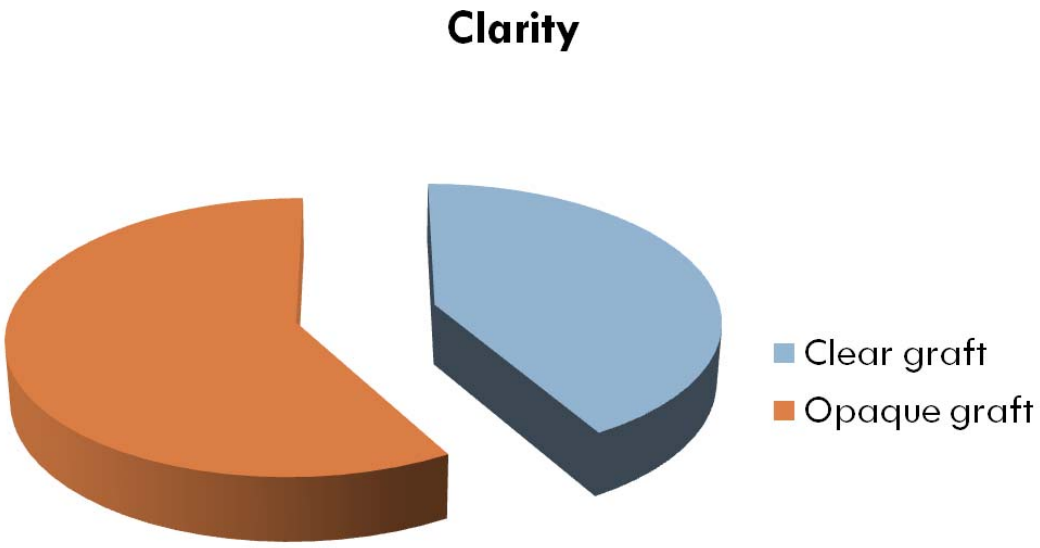
2) CORNEAL OPACITY

Status of graft

Table 5

CLEAR	OPAQUE
5	2
72 %	28%

In 1 case, due to a lack of compliance, the graft lost its clarity. In the other, there was a loss of clarity due to the occurrence of graft edema first month post-operatively.

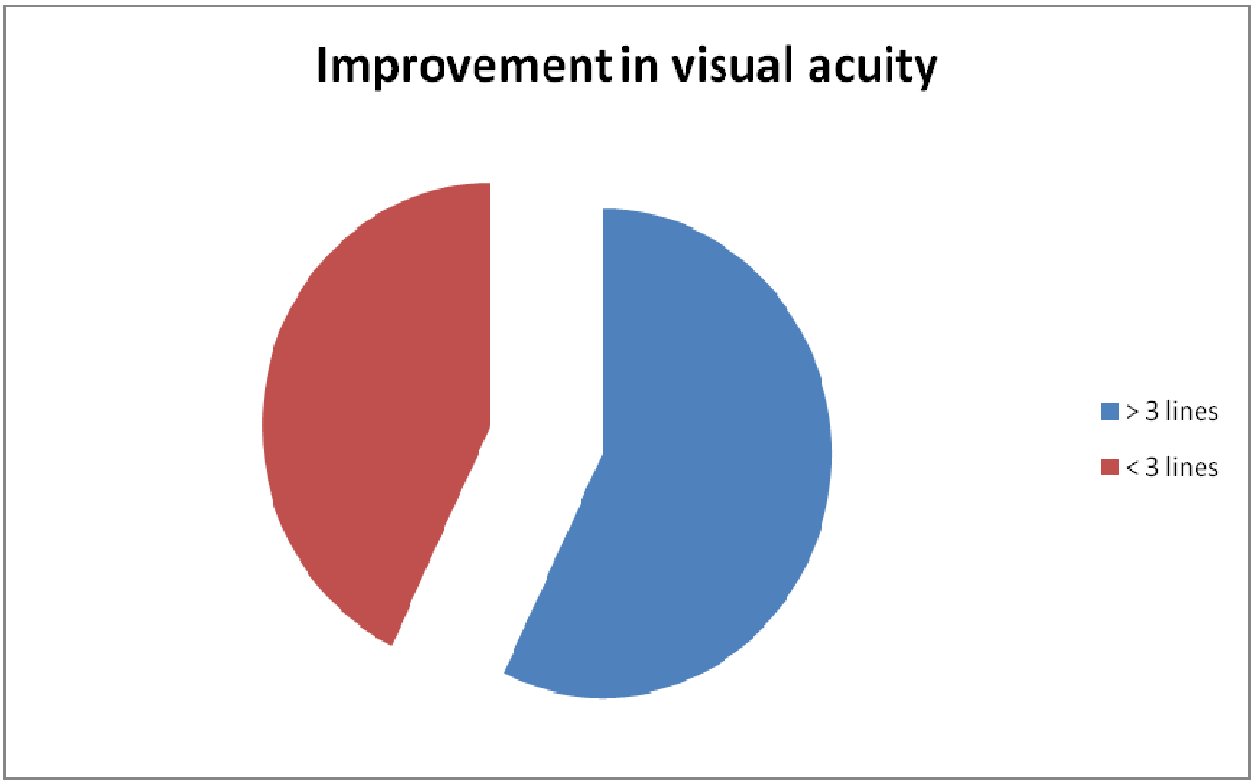


Improvement In The Visual Acuity

Table - 6

>3 lines	<3 lines
4	3
57.1 %	42.9%

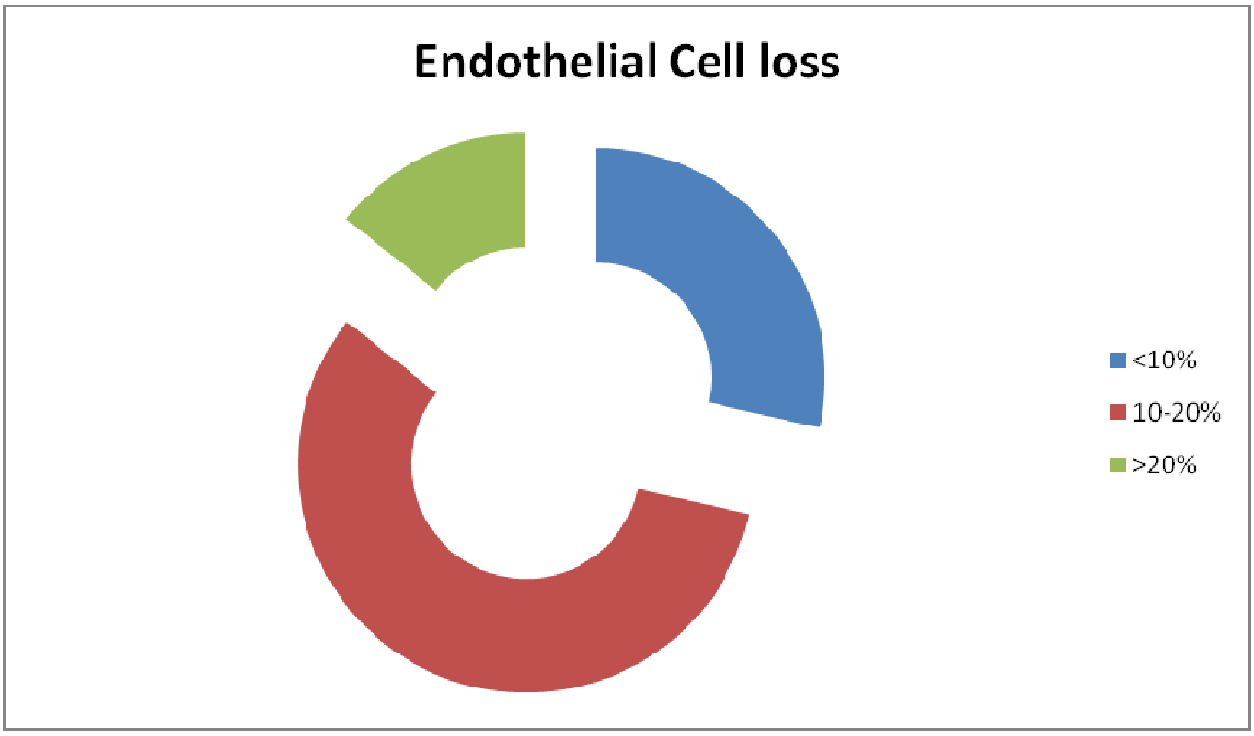
In the 3 cases that did not improve considerably, 2 had an opaque graft and 1 did not return to followup.



Endothelial Cell Loss

Table - 7

<10%	10-20%	>20%
2	4	1



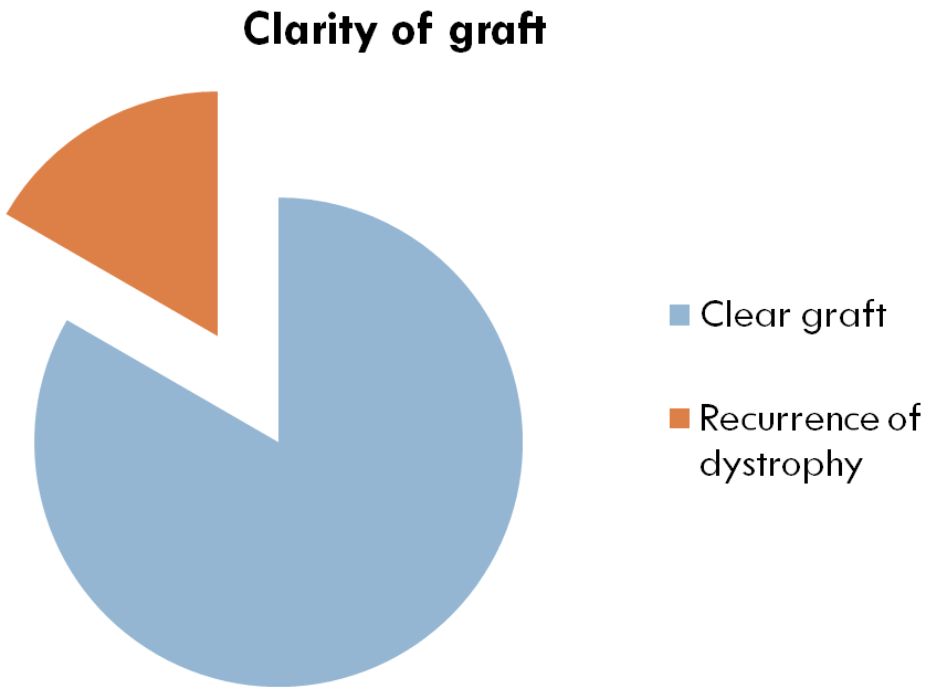
3) CORNEAL DYSTROPHY

Status of graft

Table - 8

CLEAR	RECURRENCE

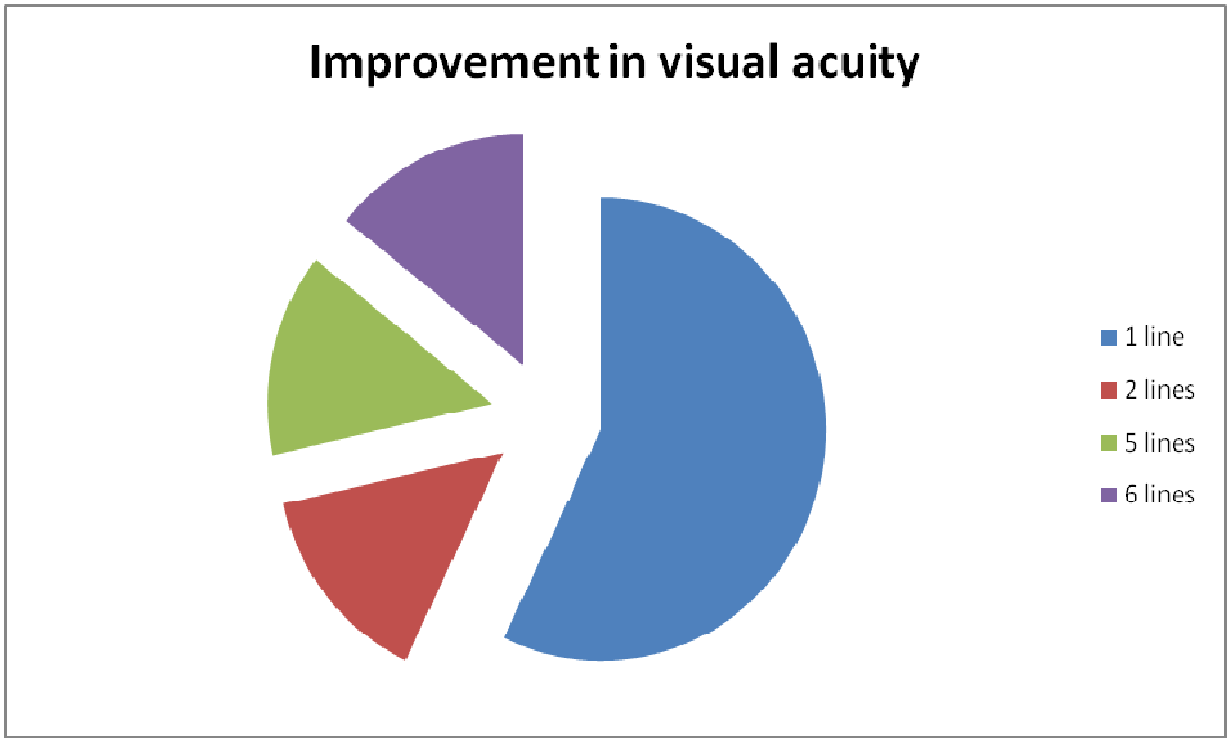
6	1
86 %	14%



Improvement in Visual Acuity

Table - 8

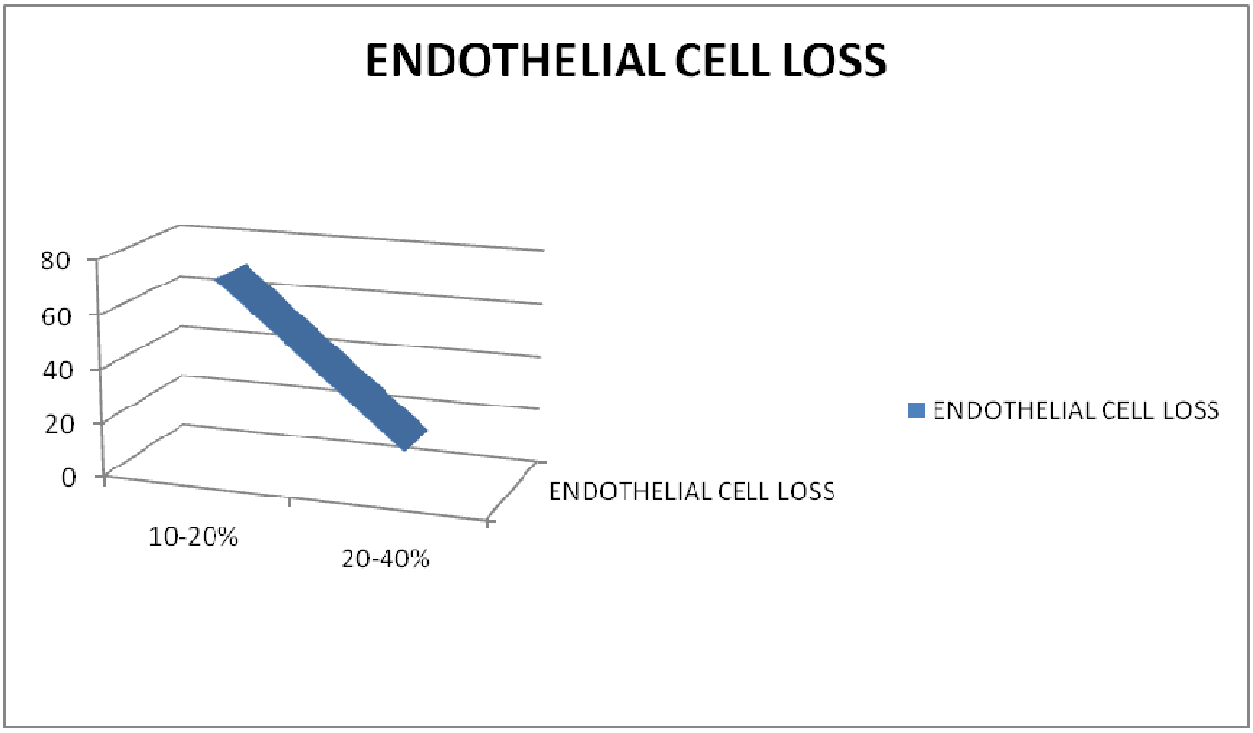
1 line	2 lines	5 lines	6 lines
4	1	1	1
57.1%	14.2%	14.2%	14.2%



Endothelial Cell Loss

Table - 9

10-20%	20-40%
5	1



4) CORNEAL DEGENERATION

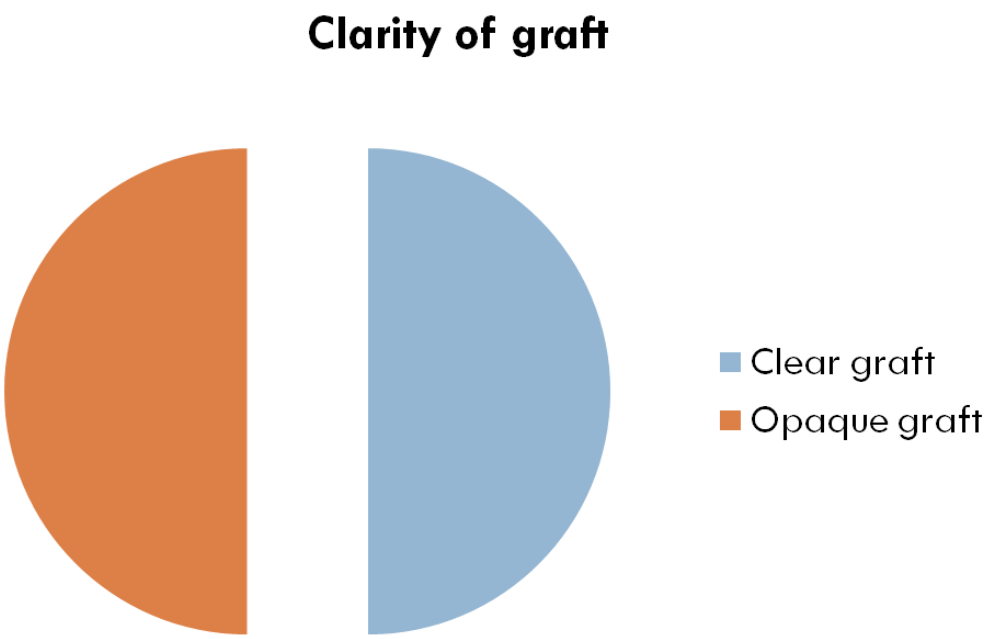
Status of the graft

Table - 10

CLEAR	OPAQUE

2	2
50%	50%

In 1 case, the graft got infected, which was treated with antiobiotics and resolved. In the other, the DALK was coupled with cataract extraction



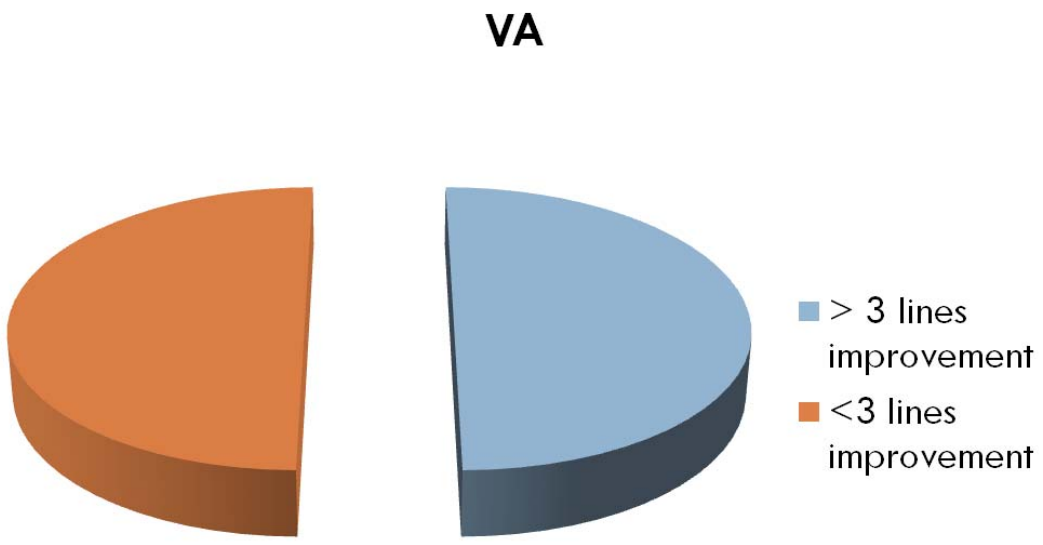
Improvement In Visual Acuity

Table - 11

>3 LINES	<3 LINES

2	2
50%	50%

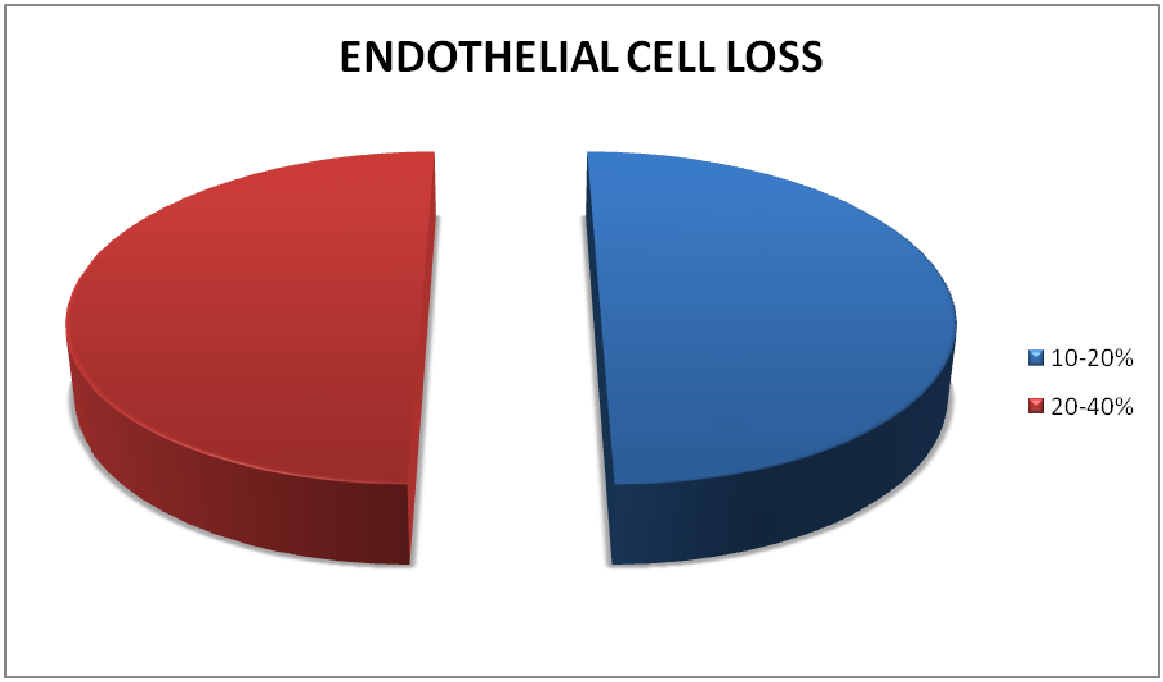
The improvement in the visual acuity was limited to 2 cases out of 4, because of the fact that the other two grafts became opaque.



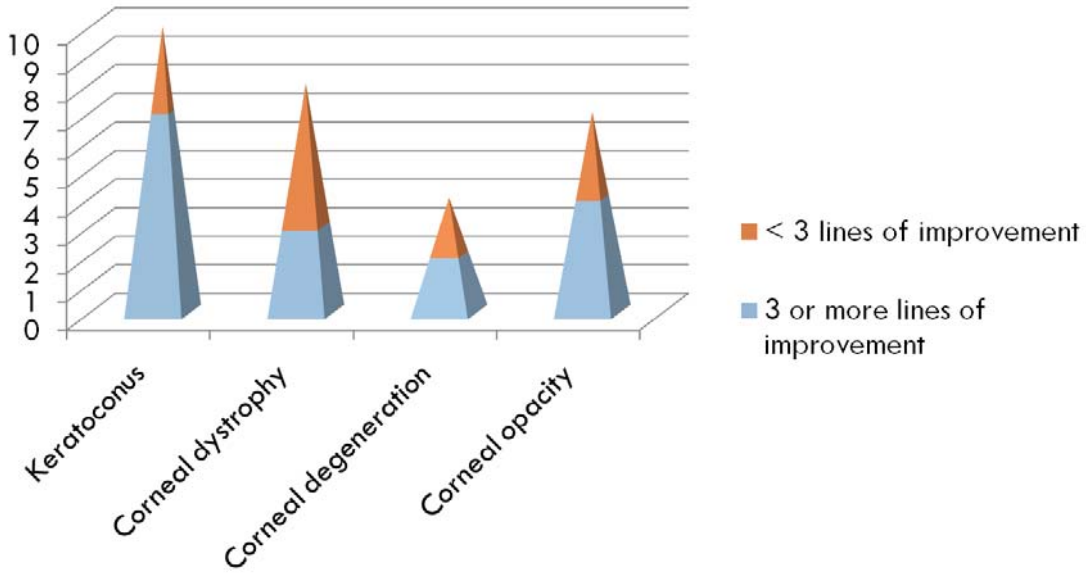
Endothelial Cell Loss

Table - 12

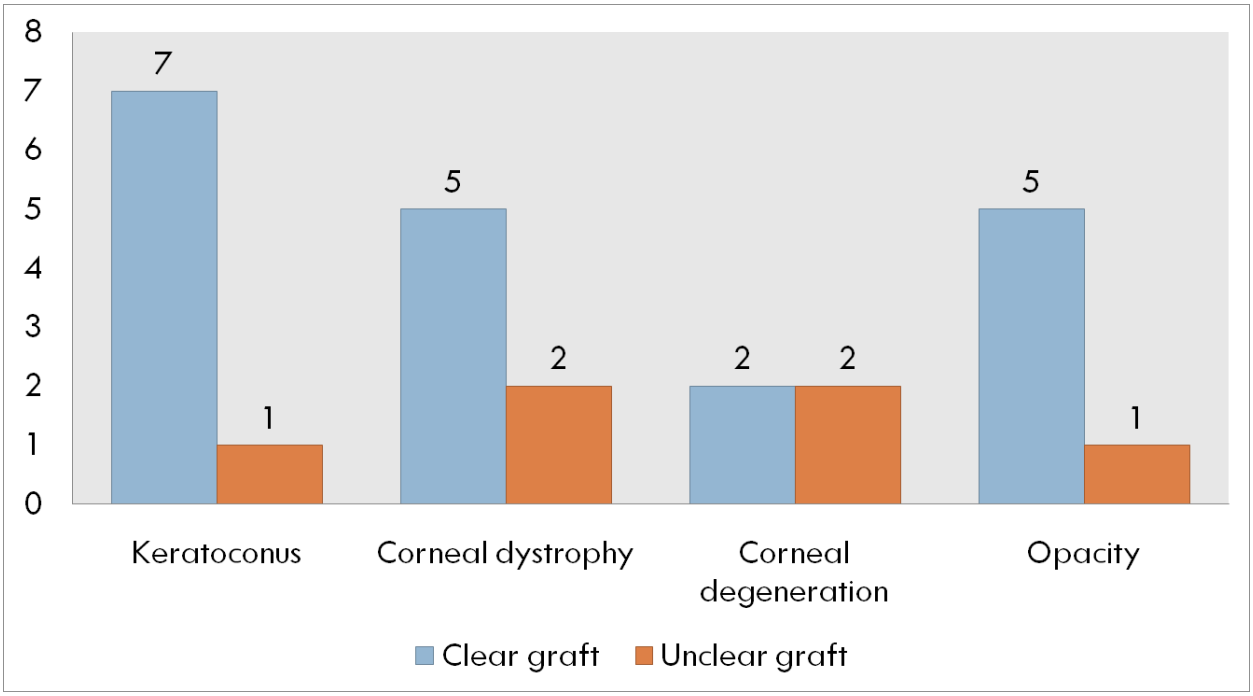
10-20%	20-40%
2	2



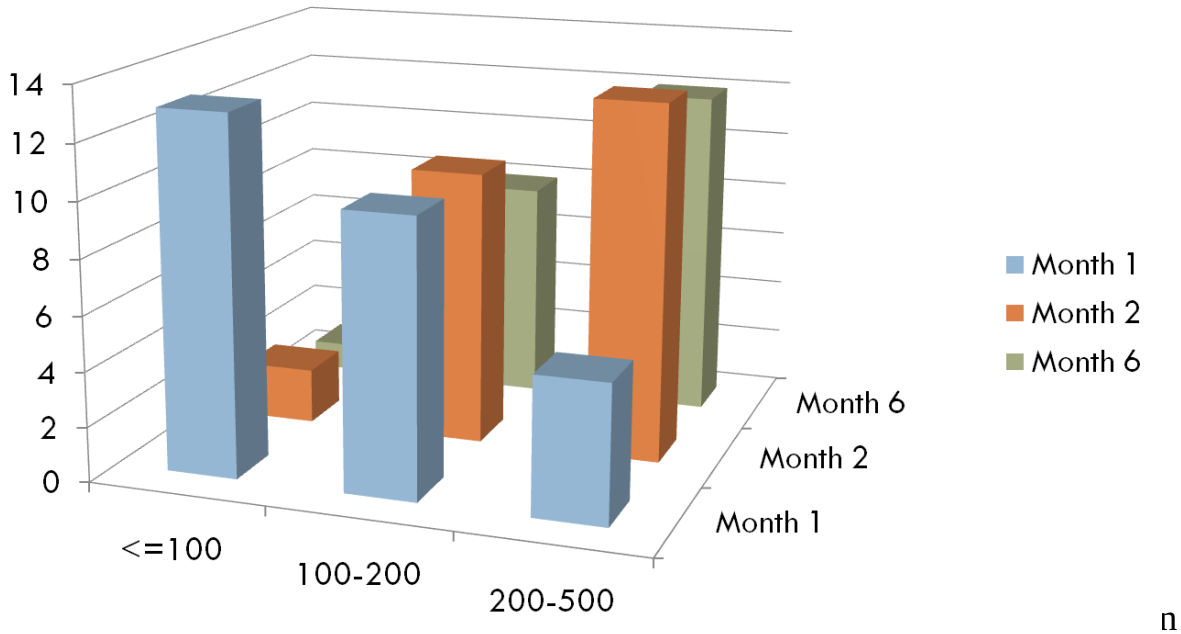
Comparison of pre and post-operative viusual acuities



Comparison of the graft clarity



The endothelial cell loss over the post-operative period



n

the first month, 44.4 % of grafts had an endothelial cell loss of less than or equal to a hundred cells. But by the sixth month, 40.7% of the grafts had an endothelial cell loss of between 200 and 500 cells At the end of one year's follow up, 51.8% of the grafts had an endothelial cell loss of between 200 and 500.

SUMMARY

Patients who underwent Deep Anterior Lamellar Keratoplasty between June 2007 and June 2009 were included in the study.

All the patients were examined pre-operatively for their Visual Acuity and Diagnosis for which the surgery was going to be done

After the surgery, the patients were examined at the end of one week. One month, 6 months and one year for their visual acuity, status of the graft and the status of the endothelium by doing a specular microscopy.

The visual acuity improvement was present in 65 % of cases that underwent the surgery. 88% of patients with keratoconus and 72% with corneal dystrophies had a clear graft at the end of the study period. The cause of graft failure was invariably lack of compliance on the part of the patient.

The endothelial cell loss was seen to progressively reduce over the study period. In the first month, 44.4 % of grafts had an endothelial cell loss of less than or equal to a hundred cells. But by the sixth month, 40.7% of the grafts had an endothelial cell loss of between 200 and 500 cells At the end of one years' follow up, 51.8% of the grafts had an endothelial cell loss of between 200 and 500.

DISCUSSION

DALK is an effective treatment for any pathology of the anterior cornea (epithelium, Bowman's layer and stroma) as long as the patient has an intact, functioning endothelium. Young patients are especially good candidates for Deep anterior Lamellar keratoplasty because they have a healthy endothelium. Hence, patients with keratoconus are good candidates for DALK because they are typically young and have healthy endothelium. Immunological rejection is an issue with Penetrating Keratoplasty, which is not so with Deep Anterior Lamellar Keratoplasty, since the patient's endothelium is left intact. In cases with corneal dystrophies, there is a risk of recurrence of the underlying disease in the graft.

But, as is the case with contact lens wear, there is a continued endothelial Cell loss after Deep Anterior Lamellar Keratoplasty.

The viability of the graft was found to be excellent after Deep Anterior Lamellar Keratoplasty. This was seen especially in cases operated for Keratoconus and Corneal dystrophies involving the anterior 2/3rds of the cornea. In our study, 87.5% of the cases with keratoconus, and 100% of those with corneal dystrophies, had a clear graft after one year of follow up.

The improvement in the visual acuity was very good after Deep anterior Lamellar Keratoplasty. The best improvement of visual acuity was found in cases who underwent the procedure for keratoconus, with 37.5 % of the cases having an

improvement of 4 lines or more. This was followed by cases who underwent the procedure for Corneal dystrophies, with 28.5 % of the cases having an improvement of 4 lines or more.

The endothelial cell loss over a period of one year was consistent in all cases that underwent Deep Anterior Lamellar Keratoplasty, though there was not any undue post-operative inflammation The maximum endothelial cell loss was found to be in patients who underwent DALK for corneal degenrations or healed corneal opacities.

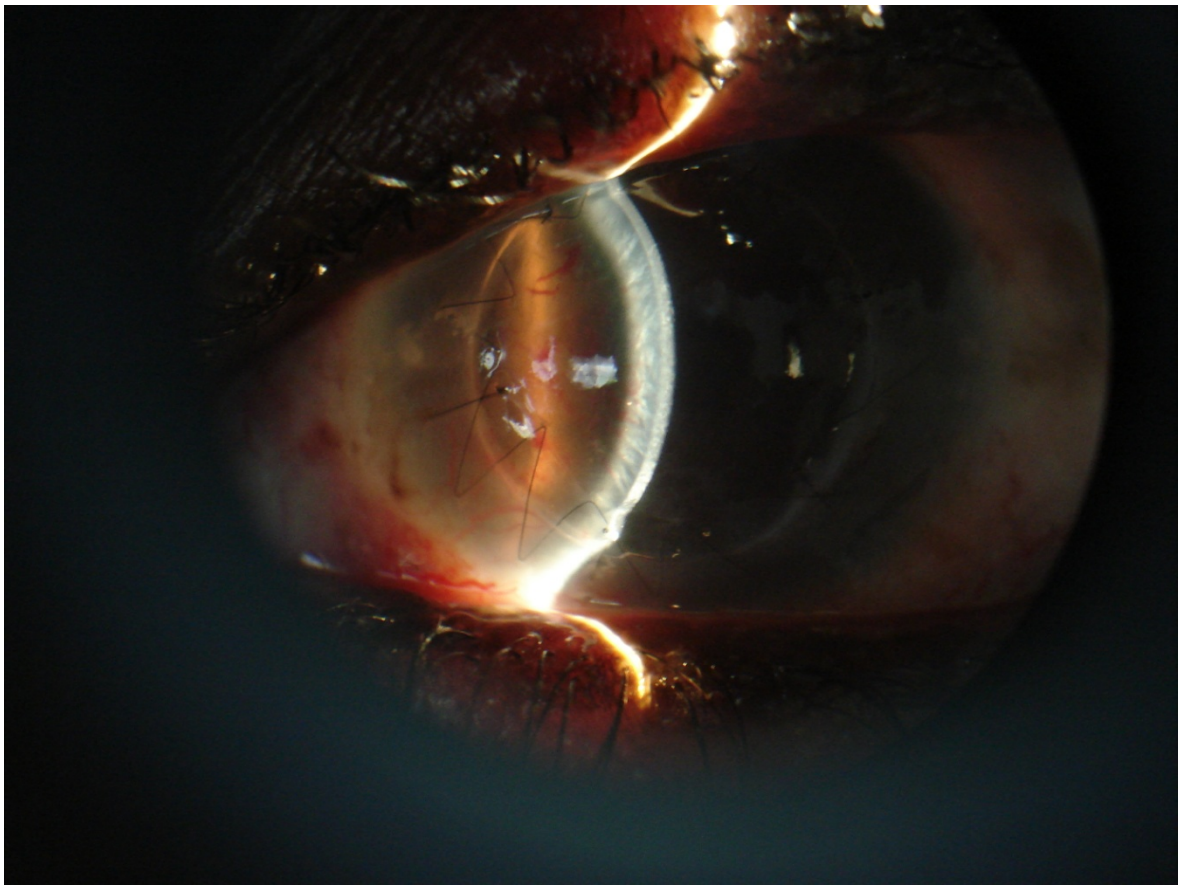
CONCLUSION

Our study proves that Deep Anterior Lamellar Keratoplasty is indeed an excellent procedure for anterior corneal lesions.The prognosis, in terms of visual acuity and viability of the graft is very good. The major cause of graft failure was lack of compliance on the part of the patient. The other causes were recurrence in case of dystrophies and graft edema due to the presence of an unhealthy endothelium pre-operatively.

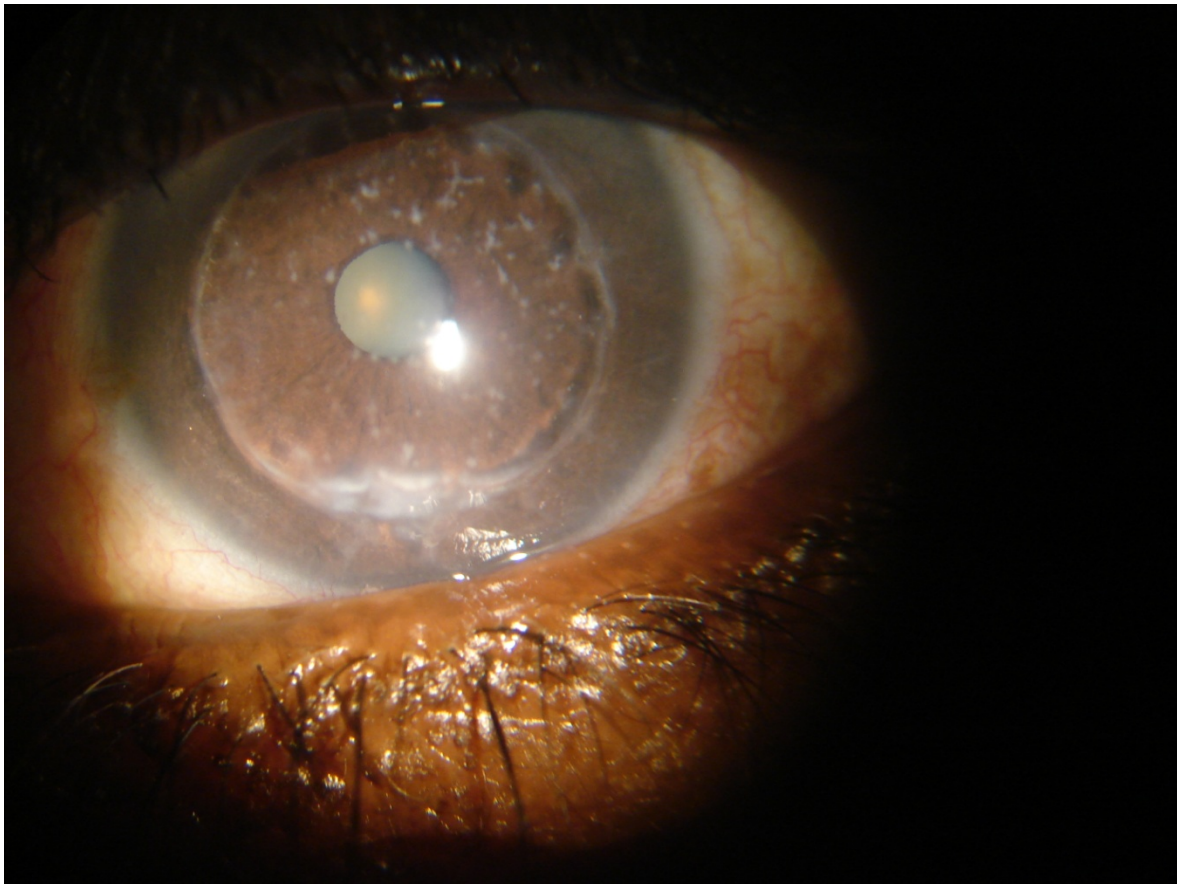
The prognosis in terms of visual acuity was also found to be excellent, especially in cases of keratoconus and corneal dystrophy.

Though the procedure does not involve any manipulation within the anterior chamber which could jeopardize the health of the corneal endothelium, there is a continued endothelial cell loss after Deep Anterior Lamellar Keratoplasty. Therefore, though it has an advantage over Penetrating keratoplasty, in terms of less chances of endothelial rejection, there is still a continued endothelial cell loss after Deep Anterior Lamellar Keratoplasty.

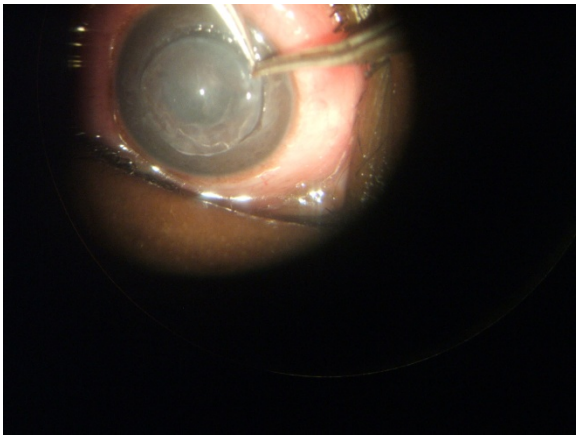
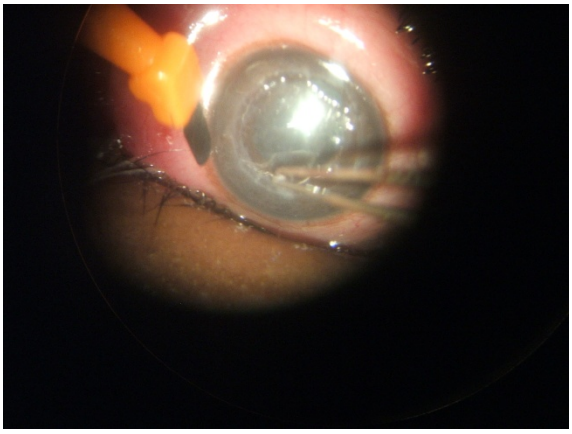
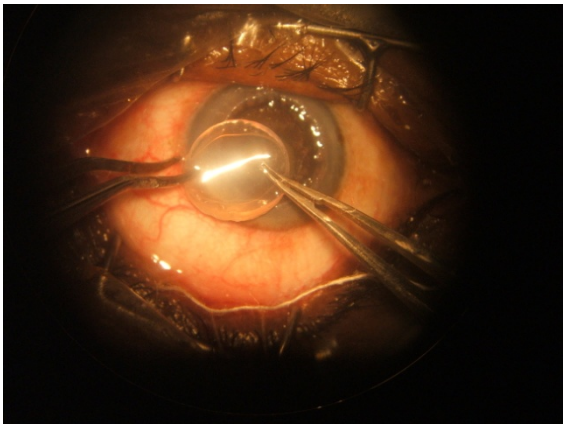
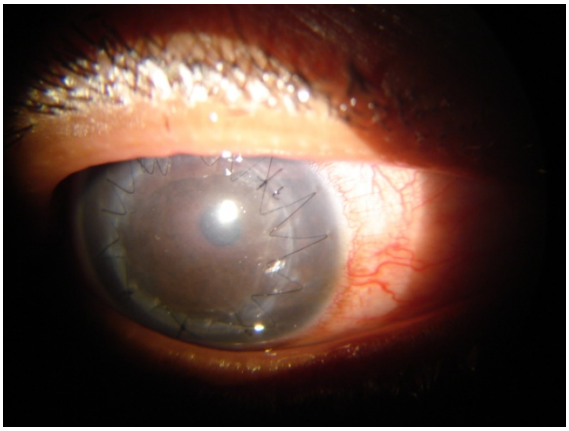
INTERFACE DEPOSITS AFTER DEEP ANTERIOR LAMELLAR KERATOPLASTY



RECURRENCE OF DYSTROPHY AFTER DEEP ANTERIOR LAMELLAR KERATOPLASTY



DEEP ANTERIOR LAMELLAR KERATOPLASTY

PROFORMA

Name

Date

Address

Age

Sex

Date of Admission

Date of Discharge

I.P. no.

Complaints: Dimuntion of vision- Duration, progression, presence/absence of pain, redness.

Present History

Past History

Family History- any similar illness in the family

History of Diabetes mellitus, hypertension, bronchial asthma

General Examination

Local Examination (both eyes)	**RE**	**LE**
Visual Axis		
Uncorrected and Best corrected visual acuity		
Lids and Adnexa		
Conjunctiva		
Cornea- depth of involvement, presence/absence of inflammation		
Anterior Chamber-presence/absence of inflammation		
Iris-presence/absence of adherence to corneal pathology		
Lens		
Pupil		
Fundus		
Intraocular Pressure		
Schirmer’s test		
Tear film Breakup time		
Specular microscopy		
Random Blood sugar		
Blood Pressure		
Urine- albumin, sugar		
Syringing of Nasolacrimal duct		

At each visit after surgery

Visual acuity- Uncorrected visual acuity

Best corrected visual acuity

Thorough slit lamp examination to assess the status of the graft

Specular microscopy to assess status of the endothelium

KEY TO MASTER CHART

LE	-	LEFT EYE
RE	-	RIGHT EYE
BE	-	BOTH EYES
VA	-	VISUAL ACUITY
PH	-	PIN HOLE
NIP	-	NOT IMPROVING WITH PINHOLE
CD	-	CELL DENSITY
DALK	-	DEEP ANTERIOR LAMELLAR KERATOPLASTY

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LIST OF SURGERIES PERFORMED

S. No.	Name	Age	Sex	IP no.	Diagnosis	Surgeries
1.	Kanniammal	60	F	684392	BE IMC	LE ECCE with PCIOL
2.	Chinnathai	65	F	785391	RE IMC/LE MC	LE ECCE with PCIOL
3.	Elangovan	76	M	790160	RE IMC/LE MC	LE ECCE with PI
4.	perumal	70	M	690437	RE IMC/LE MC	LE ECCE with PCIOL
5.	Jayagopal	50	M	692899	BE IMC	RE ECCE with PCIOL
6.	Subramani	57	M	793525	RE IMC/LE MC	LE ECCE with PCIOL
7.	Kaliammal	68	F	783728	BE Nuclear Cat.	RE ECCE with PCIOL
8.	Annammal	60	F	674267	BE MC	LE ECCE with PCIOL
9.	Krishnaveni	50	F	725015	BE IMC	LE SICS with PCIOL
10.	Muniyammal	65	F	816095	BE IMC	LE SICS with PCIOL
11.	Dhanalakshmi	46	F	785885	BE IMC	RE SICS with PCIOL

12.	Ramasamy	65	M	826095	BE IMC	RE SICS with PCIOL
13.	Lourdammal	50	F	656005	RE PSEUDO/ LE IMC	LE ECCE with PCIOL
14.	Gopal	65	M	776441	BE IMC	LE SICS with PCIOL
15.	Chandra	65	F	886336	RE MC/ LE PSEUDO	RE SICS with PCIOL
16.	Vellaiyan	55	M	847637	BE MC	LE ECCE with PCIOL
17.	Dasappan	35	M	87720	LE EXP. KERATITIS	LEMEDIAL TARSORRAPHY
18.	Etiappan	58	M	891721	LE-IMC	LE – SICS with PCIOL
19.	Mariappan	52	M	792726	LE-IMC	LE – SICS with PCIOL
20.	Shanthi	50	F	811986	LE- perforated corneal ulcer	LE-TKP
21.	Varadhan	46	M	820826	RE CDC	RE DCR
22.	Rajeshwari	54	F	762091	LE CDC	LE DCT
23.	Baskar	52	M	83940	RE-Mature Cataract	RE – SICS with PCIOL
24	Venkatesh	56	M	78542	LE-IMC	LE – SICS
25.	Lakshmi	72	F	84286	RE-non healing corneal ulcer	RE - TKP

MASTER CHART

Name	Age	Sex	IP No	Diagnosis	Pre-op VA	Procedure done	Post-op Specular week 1	Post-op specular month 1	Post-op specular month 2	Post-op specular month 6	Post-op specular month 12	Status of graft	Post-op VA
Iyyappan	42	M	82632	LE healed opacity	1/60 NIP	LE - DALK	CD-1439	CD-1340	CD- 1372	Graft edematous Specular- not			
Suman Raj	12	M	72311	BE Corneal Dystrophy	5/60 with PH 6/24	RE-DALK	CD-3218	CD-3116	CD-3098	CD- 3106	CD-3007	Clear graft	3/60 with PH 6/24
Ramchandran	74	M	83219	RE Central Macular Corneal Opacity	HM +	RE-DALK	CD-1568	CD-1406	CD-NOT POSSIBLE			Interface deposits	2/60 NIP
Chinamma	50	F	85467	BE- Spheroidal degeneration	4/60 with 5/60	RE-DALK	CD- 1315	CD-1300	CD-1298	CD-1289	CD-1267		
Nagarani	15	F	88932	LE-Corneal Opacity	2/60 NIP	LE-DALK	CD-3100	CD-3056	CD-2972	CD-2831	CD-2789	Clear Graft	6/60 with PH 6/24
Ramesh	17	M	71176	BE-Granular Dystrophy	1/60 NIP	RE-DALK	CD-1124	CD-1119	CD-1098	CD-1009	CD-1004	Clear graft	6/60 with PH 6/24
Angeline Linsy	20	F	56813	BE-Keratoconus	2/60 NIP	RE-DALK	CD-1078	CD-1279	CD-1265	CD-1106	CD-1075	Clear graft	3/60 NIP
Madhavan	22	M	76145	BE-Keratoconus	1/60 NIP	LE-DALK	CD-2192	CD-1876	CD-1657			Opaque graft	CFCF
Raghunathan	15	M	68322	BE-Keratoconus	2/60 with PH 3/60	RE-DALK	CD-1832	CD-1775	CD-1798	CD-1645	CD-1628	Clear graft	6/24 with PH 6/18
Aarthy	18	F	67013	BE-Keratoconus	2/60 with PH 6/60	RE-DALK	CD-2444	CD-2309	CD-2310	CD-2152	CD-1974	Clear graft	6/60 with PH 6/12
Saravanakumar	24	M	98763	BE-Keratoconus	1/60 NIP	LE-DALK	CD-2418	CD-2291	CD-2199	CD-1972	CD-1824	Clear Graft	6/18 with PH 6/12
Venkataraman	24	M	82154	BE Keratoconus	1/60 NIP	LE-DALK	CD-2992	CD-2912	CD-2678	CD-2156	CD-2143	Clear graft	2/60 with PH 6/36
Sohail Khan	14	M	83467	LE Keratoconus	6/60 NIP	LE-DALK	CD-2501	CD-not possible	CD-2011	CD-1825	CD-1764	Clear graft	6/60 with PH 6/24
Kannan	24	M	80158	LE Keratoconus	HM	LE-DALK	CD-2111	CD-1888	CD-1892	CD-1924	CD-1908	Clear graft	1/60 NIP
Gokulavaishnavi	21	F	81345	BE- Keratoconus	1/60 nip	RE-DALK	CD2988	CD-2870	CD-2655	CD-2500	CD-2499	Clear Graft	3/60 NIP
Saroja	60	F	82576	BE-Granular Dystrophy	1/60 NIP	LE-DALK	CD-2401	CD-2302	CD-2063	CD-1945		Recurrence of dystrophy	1/60 NIP NIG
Rajkamal	18	M	76599	LE-Corneal degeneration	CFCF	LE-DALK	CD-2456	CD-1892	CD-1644	CD-NOT POSSIBLE		Interface deposits	1/60 NIP NIG
Palanivel	37	M	83456	BE-Corneal Dystrophy	CFCF	LE-DALK	CD-1768	CD-1792	CD-1549	CD-1527	CD-1468	Clear graft	6/60 NIP

Arumugam	67	M	77718	RE Spheroidal Degeneration	CFCF	RE-DALK	CD-1574	CD-1566	CD1378	CD-NOT POSSIBLE		Graft opaque	No PL
Arjunan	49	M	66513	BE Corneal degeneration	6/60 NIP	LE-DALK	CD-2483	CD-2459	CD-2416	CD-2387	CD-2066	Cleatr graft	6/6 with PH 6/36
Yogalakshmi	12	F	82399	LE Limbal Dermoid	6/36 with PH 6/24	LE-DALK	CD-not possible	CD-2982	CD- 2889	CD-2800	CD-2776	Clear graft	6/36 with PH 6/24
Mumtaz	29	F	75609	BE-Corneal Dystrophy	2/60 NIP	RE-DALK	CD-2323	CD-2019	CD-1765	CD-1777	CD-1742	Epithelial erosion that	3/60 NIP
Karthik	12	M	70143	RE- Corneal Opacity	1/60 NIP	RE-DALK	CD-not possible	CD-2818	CD-2655	CD-2638	CD-2559	Clear Graft	2/60 NIP
Mustafa	50	F	89833	RE Epithelial Dystrophy	1/2/60 NIP	LE-DALK	CD-1599	Infiltrate in the graft					
Yakoob	40	M	74567	LE Central Corneal Opacity	1/60 NIP	LE-DALK	CD-1738	CD-1652	CD-1666	CD-1659	CD-1472	Clear graft	6/60 with PH 6/24
Masthan	52	M	80909	LE-Nebular opacity	3/60 NIP	LE-DALK	CD-1422	CD-1299	Graft opaque	LE Regraft PKP done			
Malathy	9	F	81234	RE- Corneal opacity	6/60 NIP	RE-DALK	CD-3104	CD-3008	CD-2997	CD-2985	CD-2862	Cleat graft	6/60 with ph 6/36